invitrogen™

pRSET/CFP, pRSET/EmGFP, pRSET/BFP Vectors

Catalog nos. V352-20, V353-20, V354-20

Rev. date: 14 December 2010
Manual part no. 25-0842
MAN0000519

User Manual

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Kit Contents and Storage

Shipping and Storage

pRSET vectors are shipped on wet ice. Upon receipt, store vectors at –20°C.

Kit Contents

All vectors are supplied as detailed below. **Store the vectors at –20°C.**

Catalog no.	Vector	Composition	Amount
V352-20	pRSET/CFP	20 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	10 µg
V353-20	pRSET/EmGFP	20 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	10 µg
V354-20	pRSET/BFP	20 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	10 µg

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Introduction

Product Overview

Description of the System

pRSET/CFP, pRSET/EmGFP and pRSET/BFP vectors are bacterial expression vectors that contain sequences encoding Fluorescent Proteins (FPs). FPs are derived from Green Fluorescent Protein, and contain amino acid substitutions that alter the spectral properties of the proteins (see page 3 for details). Upon excitation, these FPs emit a fluorescent signal corresponding to the colors cyan (CFP), emerald green (EmGFP) and blue (BFP).

The FP sequences have been cloned into the bacterial expression vector pRSET A to produce pRSET/CFP, pRSET/EmGFP and pRSET/BFP. For a description of the major features of the vectors, see page 2.

Applications

The pRSET Fluorescent Vectors may be used as follows:

- Remove the Fluorescent Protein gene from the vector by restriction digest for cloning into a mammalian expression vector of choice to create a reporter vector,

  OR

- Express the Fluorescent Protein in *E. coli,* and detect and purify the protein for further study.

Continued on next page

Product Overview, Continued

Features of pRSET Fluorescent Vectors

Features of the pRSET Fluorescent Vectors include:

- Bacteriophage T7 promoter for high-level, inducible expression of the Fluorescent Protein (FP) in *E. coli*
- Ribosome binding site (RBS) optimally spaced from the initiation ATG for efficient translation of the FP
- N-terminal fusion peptide encoding:

  6xHis tag for protein purification using metal binding resins

  Xpress™ epitope for detection of the expressed fusion protein using an Anti-Xpress™ antibody

  Enterokinase (EK) recognition site for efficient cleavage of the fusion peptide from the FP
- Fluorescent Protein derived from eGFP (CFP, EmGFP, BFP)
- Ampicillin resistance gene for selection in *E. coli*
- pUC origin for high-copy replication and maintenance of the plasmid in *E. coli*

Green Fluorescent Protein

Green Fluorescent Protein (GFP) is a chemi-luminescent protein originally isolated from the *Aequorea victoria* jellyfish (Shimomura *et al.*, 1962). GFP is a useful biotechnology tool because the gene encoding GFP contains all necessary information for the posttranslational synthesis of the chromophore.

GFP is widely used as a reporter, either when fused to a gene of interest or co-expressed in mammalian cells. The GFP fluorescence signal is easily detected using fluorescence microscopy and standard filter sets.

Modifications have been made to wild-type GFP to enhance its expression in mammalian systems. These modifications include amino acid substitutions that:

- Change the spectral properties of the protein
- Optimize the codon usage for expression in mammalian cells, *i.e.*, enhanced GFP (eGFP) (Zhang *et al.*, 1996).

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Modified Fluorescent Proteins

Fluorescent Proteins (FPs) that emit fluorescence signals at various wavelengths have been created by introducing amino acid substitutions in the eGFP protein. These mutations shift the spectral properties of the protein, resulting in cyan (CFP), emerald (EmGFP) or blue (BFP) detected fluorescence.

Mutations in the FP genes of the pRSET Fluorescent Vectors have been described in a published review (Tsien, 1998) and are summarized in the table below. These mutations are represented by the single letter amino acid abbreviation corresponding to the codon number in the consensus sequence of eGFP followed by the single letter amino acid abbreviation for the substituted amino acid.

Vector	eGFP Mutations*
pRSET/CFP	K26R, Y66W, N146I, M153T, V163A, N164H
pRSET/EmGFP	F64L, S65T, S72A, N149K, M153T, I167T
pRSET/BFP	F64L, Y66H, Y145F, V163A, N198S

*Mutations listed are as described in the literature. When examining the actual sequence, the vector codon numbering starts at the first amino acid **after** the initiation methionine of the FP, so that mutations appear to be increased by one position. For example, the F64L mutation actually occurs in codon 65 of the protein.

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Fluorescent Protein Spectral Properties

Fluorescent Proteins can be detected using fluorescence microscopy or other methods that use light excitation and detection of emission. The table below lists the published excitation and emission wavelengths for CFP, EmGFP, and BFP (Tsien, 1998).

All three FPs can be detected with standard FITC filter sets. However, for optimal detection of the fluorescence signal, you may want to use a filter set optimized for detection within the excitation and emission ranges for each FP. These filter sets are listed in the table below.

Vector	Excitation/ Emission (nm)	Filter Set for Fluorescence Microscopy
pRSET/CFP	452/505	Omega XF114 Chroma 31044
pRSET/EmGFP	487/509	Omega XF100
pRSET/BFP	308-383/ 440-447	Omega XF10 Chroma 31021

For information on obtaining filter sets, contact Omega Optical, Inc. (www.omegafilters.com) or Chroma Technology Corporation (www.chroma.com).

Methods

General Information

Applications

You may use the pRSET Fluorescent Vectors for the following applications:

- Transfer the Fluorescent Protein gene into a mammalian vector of choice using restriction enzyme cloning. See below for guidelines.
- Express the Fluorescent Protein in *E. coli* and purify the protein. See page 7 for guidelines.

E. coli Host for Vector Propagation

To propagate and maintain pRSET Fluorescent Vectors, we recommend using a *recA*, *endA* strain such as One Shot® TOP10F′ (see page 11 for ordering) or DH5α. Select plasmid-containing transformants on LB plates containing 50-100 µg/mL ampicillin.

Plasmid Purification

You may prepare plasmid DNA using your method of choice. We recommend using the PureLink™ HiPure Plasmid Purification Kit (see page 11).

Transferring the FP Gene to Another Vector

Each pRSET/FP Vector contains unique restriction sites flanking the FP gene to allow transfer of the FP gene to any vector of choice (e.g. mammalian expression vector) using restriction enzyme cloning. Refer to the vector map on page 8 to develop your cloning scheme.

Note: If you clone the FP gene into a mammalian vector, remember to include a Kozak consensus sequence for proper translation initiation. If you are creating a fusion vector, remember to clone the FP gene in-frame with the gene of interest.

Continued on next page

Expression of Fluorescent Protein in *E. coli*

Introduction

The pRSET Fluorescent Vectors allow expression of the Fluorescent Protein gene in *E. coli* under the control of the strong bacteriophage T7 promoter. The following section provides guidelines for choosing an appropriate *E. coli* strain for transformation of the pRSET Fluorescent Vector and for induction of Fluorescent Protein expression.

Important

We recommend that you maintain and propagate the plasmid in a *recA*, *endA* strain of *E. coli* such as TOP10 or DH5α. **Do not propagate your vector in a BL21 strain of *E. coli*.**

E. coli Host for Protein Expression

In bacteriophage T7, the T7 promoter drives the expression of gene 10. T7 RNA polymerase recognizes this promoter. To express the Fluorescent Protein gene in *E. coli*, you may use a bacterial host that expresses T7 RNA polymerase or infect the cell with phage expressing T7 RNA polymerase.

We suggest using a BL21-derived *E. coli* strain as the host for the expression construct. These strains express T7 RNA polymerase in a regulated manner.

Induction of Protein Expression

For isopropyl-D-thiogalactoside (IPTG) induction of protein expression, use a BL21-derived strain that contains the DE3 bacteriophage λ lysogen. The DE3 lysogen contains the T7 RNA polymerase under the control of the *lacUV5* promoter, allowing expression of T7 RNA polymerase to be induced by IPTG.

We recommend using BL21 Star™(DE3) available from Invitrogen (see page 11) This strain contains the bacteriophage λ DE3 lysogen. Refer to the user manual for the strain you are using for detailed instructions on expressing protein.

Purification and Detection of Fluorescent Protein

Introduction

Once you have expressed the FP fusion protein, you may verify expression by simply holding the bacterial cell lysate under a UV light source to detect the fluorescent signal. Alternatively, you may perform western blot analysis to detect the fusion protein using the antibodies listed below. Guidelines for protein purification can also be found below.

Detection Methods

You may detect expression of your recombinant fusion protein by western blot using anti-Xpress™ and anti-GFP antibodies (see page 11).

Purification Guidelines

The presence of the polyhistidine (6xHis) tag in the fusion peptide of the Fluorescent Protein allows the use of a metal-chelating resin such as ProBond™ or Ni-NTA to purify your fusion protein. ProBond™ and Ni-NTA are available from Invitrogen (see page 11 for ordering). Refer to the manual included with each product for instructions to purify your 6xHis-tagged fusion protein.

Note: Other metal-chelating resins and purification methods are suitable.

Cleavage of the Fusion Peptide by Enterokinase

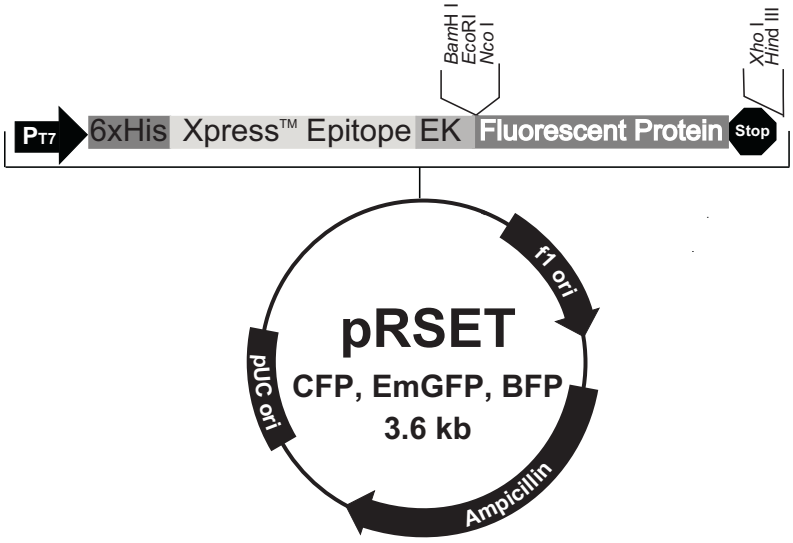
The pRSET Fluorescent Vectors contain an Enterokinase (EK) recognition site to allow removal of the fusion tag from the expressed FP. We recommend using EnterokinaseMax™ from Invitrogen; see page 11 for ordering information.

Appendix

pRSET/CFP, EmGFP and BFP Vectors

Map of pRSET Vectors

The map below shows the features of pRSET/CFP, pRSET/EmGFP, and pRSET/BFP. Note that the vectors are identical in size, and only differ from one another in the sequence of the Fluorescent Protein gene. The vector sequence is available for downloading at www.invitrogen.com or by contacting **Technical Support** (page 12).



Comments for pRSET/CFP, EmGFP and BFP
3600 nucleotides

T7 promoter/priming site: bases 9-28
6xHis tag: bases 101-118
T7 gene 10 leader: bases 122-154
Xpress™ epitope: bases 158-181
EK cleavage site: bases 167-181
Fluorescent Protein (CFP, EmGFP, BFP): bases 209-928
T7 reverse priming site: bases 987-1006
T7 transcription terminator: bases 948-1084
f1 origin: bases 1148-1603
bla promoter: bases 1635-1739
Ampicillin (bla) resistance gene: bases 1734-2594
pUC origin: bases 2739-3412

Continued on next page

pRSET/CFP, EmGFP, and BFP Vectors, Continued

Features

The pRSET/CFP, pRSET/EmGFP and pRSET/BFP vectors (3654 bp) contain the following elements. Elements have been functionally tested.

Feature	Function
T7 promoter	Provides tight, dose-dependent regulation of heterologous gene expression.
T7 forward priming site	Allows sequencing of the insert.
Ribosome binding site (RBS)	Optimally spaced from the cloning region for efficient translation of the gene of interest.
Initiation ATG	Provides a translational initiation site for the fusion protein.
6XHis Tag	Permits purification of recombinant fusion protein on metal chelating resins such as ProBond™ and Ni-NTA.
T7 gene 10 leader	Provides protein stability.
Xpress™ epitope	Allows detection of the fusion protein using an anti-Xpress™ antibody.
Enterokinase (EK) recognition site	Provides a site for efficient removal of the fusion tag using EKMax™.
Fluorescent Protein (CFP, EmGFP or BFP)	Modified fluorescent proteins derived from eGFP, whose expression results in the emission of fluorescent signal.
T7 reverse priming site	Allows sequencing of the insert.
T7 terminator	Permits efficient transcription termination.
f1 origin	Allows single strand rescue of DNA.
bla promoter	Allows expression of the ampicillin resistance gene.
Ampicillin ORF	Allows selection of the plasmid in *E. coli.*
pUC origin	Allows high copy replication and growth in *E. coli.*

Recipes

LB Medium and Plates

LB medium (1 Liter)

1. Dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 mL deionized water.
2. Adjust the pH of the solution to 7.0 with NaOH and bring the volume up to 1 liter.
3. Autoclave on liquid cycle for 20 minutes. Allow solution to cool to ~55°C, and add antibiotic if needed.
4. Store at room temperature or at 4°C.

LB agar plates

1. Prepare LB medium as described above, but add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle for 20 minutes.
3. Allow agar to cool to ~55°C, and add antibiotic. Pour 20–30 mL agar into each 10 cm plate.
4. Let agar harden, then invert and store at 4°C, in the dark.

Glycerol Stocks

1. Streak a colony out for single colony isolation on LB plates containing the appropriate antibiotic.
2. Isolate a single colony and inoculate into 1–2 mL of LB containing the appropriate antibiotic.
3. Grow until culture reaches stationary phase.
4. Mix 0.85 mL of culture with 0.15 mL of sterile glycerol and transfer to a cryovial.
5. Store at –80°C.

Accessory Products

Introduction

The following products may be used with the pRSET vectors. For details, visit www.invitrogen.com or contact **Technical Support** (see page 12).

Item	Amount	Catalog no.
ProBond™ Purification System	6 × 2 mL precharged, prepacked ProBond™ resin columns and buffers for native and denaturing purification	K850-01
ProBond™ Resin	50 mL	R801-01
Electrocomp™ TOP10F´	150 mL	R801-15
	6 × 20 rxns	C665-24
One Shot® TOP10F´ Chemically Competent *E. coli*	20 × 50 µL	C3030-03
BL21 Star™(DE3)	20 rxns	C6010-03
PureLink™ HiPure Plasmid Miniprep Kit	100 preps	K2100-03
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-04
EnterokinaseMax™	250 units	E180-01
Anti-Xpress™ Antibody	50 µL	R910-25
Anti-GFP (rabbit polyclonal)	100 µL	A11122
Anti-GFP (rabbit IgG conjugated to Alexa Fluor® 488)	100 µL	A21311
Anti-GFP (mouse monoclonal IgG)	100 µg	A11120

Technical Support

Web Resources

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

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Technical Support, Continued

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