invitrogen™
by life technologies™

pRSET A, B, and C

For high-level expression of recombinant proteins in *E. coli*

Cat. no . V351-20

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Manual part no. 25-0213

MAN00000061

User Manual

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Kit Contents and Storage

Kit Contents

This kit contains the following reagents:

20 µg each of pRSET A, B, and C in TE buffer, pH 8.0* (40 µl each at 0.5 µg/µl)

1 stab TOP10F′

1 stab BL21(DE3)pLysS

1 stab BL21(DE3)pLysS containing the pRSET/*lac*Z control

***TE buffer, pH 8.0:** 10 mM Tris-HCl, 1 mM EDTA, pH 8.0

Shipping and Storage

This kit is shipped on wet ice. Upon receipt, store the plasmids at –20°C and the stabs at 4°C.

Long-Term Storage

For long-term storage of *E. coli* strains supplied as stabs with this kit, prepare glycerol stocks as follows:

1. Grow the *E. coli* strain overnight in SOB medium overnight with antibiotic selection when appropriate.
2. Combine 0.85 ml of the overnight culture with 0.15 ml of sterile glycerol.
3. Vortex and transfer to a labeled cryovial.
4. Freeze the tube in liquid nitrogen or dry ice/ethanol bath and store at –80°C.

Accessory Products

Introduction

The tables below lists related products that may be used with pRSET A, B, and C.

Product	Application	Quantity	Cat. No.
One Shot® TOP10F′ cells	Chemically competent cells for transformation	20 × 50 µl	C3030-03
One Shot® BL21(DE3)pLysS cells	Chemically competent cells for transformation	20 × 50 µl	C6060-03
One Shot® BL21(DE3)pLysE cells	Chemically competent cells for transformation	20 × 50 µl	C6565-03
One Shot® BL21(DE3) cells	Chemically competent cells for transformation	20 × 50 µl	C6000-03
Anti-Xpress™ Antibody	Detection of recombinant proteins	50 µl	R910-25
Anti-Xpress™-HRP Antibody	Detection of recombinant proteins	50 µl	R911-25
Anti-HisG Antibody	Detection of recombinant proteins	50 µl	R940-25
Anti-HisG-HRP Antibody	Detection of recombinant proteins	50 µl	R941-25
Anti-HisG-AP Antibody	Detection of recombinant proteins	125 µl	R942-25
ProBond™ Resin	Purification of recombinant proteins	50 ml	R801-01
		150 ml	R801-50
EnterokinaseMax™	Removal of N-terminal peptide	250 units	E180-01
EK-Away™	Removal of EnterokinaseMax™	7.5 ml	R180-01
ProBond™ Purification System	For native and denaturing purification of recombinant proteins	6 purifications	K850-01

Continued on next page

Accessory Products

Electrophoresis Products

A large variety of pre-cast polyacrylamide gels and electrophoresis products are available separately from Invitrogen for the separation and analysis of recombinant proteins. Ordering information for the most widely used products is provided below. For more detailed information, including size, concentration and well formats available for pre-cast gel systems, visit www.invitrogen.com or contact Technical Support (page 18).

Product	Quantity	Cat. no.
NuPAGE® Novex® 4–12% Bis-Tris Gels	1 box (10 gels)	NP0321BOX
Novex® 10% Tris-Glycine Gels	1 box (10 gels)	EC6075BOX
NuPAGE® LDS Sample Buffer (4X)	10 ml 250 ml	NP0007 NP0008
Novex® Tris-Glycine SDS Sample Buffer (2X)	20 ml	LC2676
SimplyBlue™ Safe-Stain	1 L	LC6060
Colloidal Blue Staining Kit	1 kit	LC6025
XCell *SureLock*™ Mini-Cell & XCell II™ Blot Module	1 unit	EI0002

Media and Reagents

In addition to the pre-cast polyacrylamide gel systems, Invitrogen offers a wide range of pre-mixed media and reagents. Ordering information for the most widely used products is provided below. For more detailed information, visit www.invitrogen.com or contact Technical Support (page 18).

Product	Quantity	Cat. no.
S.O.C. Medium	10 × 10 ml	15544-034
(Miller's LB Broth Base) ® Luria Broth Base, powder	500 g	12795-027
imMedia™ Amp Liquid	20 pouches (200 ml medium)	Q600-20
imMedia™ Amp Agar	20 pouches (8–10 plates)	Q601-20
UltraPure™ Sodium Dodecyl Sulfate (SDS)	500 g	15525-017
UltraPure™ DNase/RNase-Free Water	500 ml	10977-015

Introduction

Overview

Introduction

The pRSET vectors are pUC-derived expression vectors designed for high-level protein expression and purification from cloned genes in *E. coli*. High levels of expression of DNA sequences cloned into the pRSET vectors are made possible by the presence of the T7 promoter. In addition, DNA inserts are positioned downstream and in frame with a sequence that encodes an N-terminal fusion peptide. This sequence includes an ATG translation initiation codon, a polyhistidine tag that functions as a metal binding domain in the translated protein, a transcript stabilizing sequence from gene 10 of phage T7, the Xpress™ epitope, and the enterokinase cleavage recognition sequence.

The metal binding domain of the fusion peptide allows simple purification of recombinant proteins by Immobilized Metal Affinity Chromatography with Invitrogen's ProBond™ resin (available in bulk, see page v). The enterokinase cleavage recognition site in the fusion peptide located between the metal binding domain and the recombinant protein allows for subsequent removal of this N-terminal fusion peptide from the purified recombinant protein.

Regulation of Expression of the Gene of Interest

Expression of the gene of interest from pRSET is controlled by the strong phage T7 promoter that drives expression of gene 10 (Φ10). T7 RNA polymerase specifically recognizes this promoter. For expression of the gene of interest, it is necessary to deliver T7 RNA polymerase to the cells by either inducing expression of the polymerase using the gratuitous inducer isopropyl β-D-thiogalactoside (IPTG), or infecting the cell with phage expressing the polymerase. Once sufficient T7 RNA polymerase is produced, it binds to the T7 promoter and transcribes the gene of interest.

Regulation of Expression of T7 RNA Polymerase

The BL21(DE3)pLysS strain is specifically included in the kit for expression of T7 regulated genes. This strain carries the DE3 bacteriophage lambda lysogen. This lambda lysogen contains the *lac*I gene, the T7 RNA polymerase gene under control of the *lac*UV5 promoter, and a small portion of the *lac*Z gene. This *lac* construct is inserted into the *int* gene, which inactivates the *int* gene. Disruption of the *int* gene prevents excision of the phage (i.e. lysis) in the absence of helper phage. The *lac* repressor represses expression of T7 RNA polymerase. Addition of IPTG allows expression of T7 RNA polymerase.

The BL21(DE3)pLysE strain is also available. For more information on this strain, BL21(DE3), and BL21(DE3)pLysS, see page 3.

Continued on next page

Overview, continued

Regulation of T7 RNA Polymerase by T7 Lysozyme

There is always some basal level expression of T7 RNA polymerase. If a toxic gene is cloned downstream of the T7 promoter, basal expression of this gene may lead to reduced growth rates, cell death, or plasmid instability. T7 lysozyme (produced from pLysS or pLysE) has been shown to bind to T7 polymerase and inhibit transcription. This activity is exploited to reduce basal levels of T7 RNA polymerase.

T7 lysozyme is a bifunctional enzyme. In addition to its T7 RNA polymerase binding activity, it also cleaves a specific bond in the peptidoglycan layer of the *E. coli* cell wall. This activity increases the ease of cell lysis by freeze-thaw cycles prior to purification.

Experimental Outline

The table below describes the basic steps needed to clone and express your protein using pRSET A, B, and C. For more details, please refer to the page(s) indicated.

Step	Action	Page
1	Propagate and maintain the empty pRSET A, B, and C vectors by transforming them into a *rec*A, *end*A *E. coli* host (i.e. TOP10F′).	3
2	Develop a cloning strategy to ligate your gene of interest into pRSET A, B, or C.	4–7
3	Ligate your gene of interest into pRSET, transform into TOP10F′, and select on 50–100 µg/ml ampicillin.	6
4	Sequence your construct to ensure that it is in frame with the N-terminal peptide.	7
5	Perform a pilot expression using IPTG for induction.	8
6	Purify your recombinant protein by chromatography on metal-chelating resin (e.g. ProBond™).	11

Methods

General Cloning

Introduction

The following information is provided to help you clone your gene of interest into pRSET A, B, and C. For basic information on DNA ligations, *E. coli* transformations, restriction analysis, DNA sequencing and DNA biochemistry, see *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

E. coli Host

For cloning and transformation, we recommend using a *rec*A, *end*A strain such as TOP10F′ (included in the kit). TOP10F′ cells are *recA* and *endA* making them suitable for cloning, propagation, and maintenance.

Genotype of TOP10F′:

F′ {*lac*I^q, Tn*10*(Tet^R)} *mcr*A (*mrr-hsd*RMS-*mcr*BC) 80*lac*Z M15 *lac* 74 *rec*A1 *ara*D139 (*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G.

BL21(DE3)pLysS is specifically designed for expression of genes regulated by the T7 promoter. Do not use this strain for propagation or maintenance of your plasmid.

Genotype of BL21(DE3)pLysS:

F$^-$, *omp*T *hsd*SB (r_B^- m_B^-) *gal dcm* (DE3) pLysS (Cam^R)

Maintaining pRSETA, B, and C

To propagate and maintain pRSET A, B, and C, use the supplied 0.5 µg/µl stock solution in TE, pH 8.0 to transform a *rec*A, *end*A *E. coli* strain like TOP10F′, DH5α™-T1 ^{R}, TOP10, or equivalent. Select transformants on LB plates containing 50–100 µg/ml ampicillin.

Be sure to prepare a glycerol stock of a transformant containing plasmid for long-term storage (see page 7).

Cloning into pRSET A, B, and C

Introduction

The multiple cloning site of each version of pRSET is provided below and on the following pages (see pages 5–6). To generate recombinant proteins that are expressed correctly and contain the N-terminal fusion peptide, it is necessary to clone in frame with the N-terminal peptide. To facilitate cloning, the pRSET vector is provided in three different reading frames. They differ only in the spacing between the sequences that code for the N-terminal peptide and the multiple cloning site. For proper expression, determine which restriction sites are appropriate for ligation.

Multiple Cloning Site of pRSET A

Below is the multiple cloning site for pRSET A. Restriction sites are labeled to indicate the actual cleavage site. The boxed nucleotides indicate the variable region. Sequencing and functional testing have confirmed the multiple cloning site. The complete sequence of pRSET A is available for downloading at www.invitrogen.com or from Technical Support (see page 18). For a map and description of the features of pRSET A, please refer to pages 14–15.

T7 promoter — RBS

```
21   AATACGACTC ACTATAGGGA GACCACAACG GTTTCCCTCT AGAAATAATT TTGTTTAACT TTAAGAAGGA
```

Polyhistidine (6xHis) region

```
91   GATATACAT ATG CGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG ACT
               Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
```

T7 gene 10 leader — Xpress™ Epitope — *Bam*H I

```
148  GGT GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT AAG GAT CGA TGG GGA
     Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp Arg Trp Gly
```

EK recognition site — EK cleavage site

Xho I *Sac* I *Bgl* II — *Pst* I *Pvu* II — *Kpn* I *Nco* I — *Eco*R I — *Bst*B I — *Hin*d III

```
205  TCC GAG CTC GAG ATC TGC AGC TGG TAC CAT GGA ATT CGA AGC TTG ATC CGG CTG CTA
     Ser Glu Leu Glu Ile Cys Ser Trp Tyr His Gly ile Arg Ser Leu Ile Arg Leu Leu
```

T7 reverse priming site

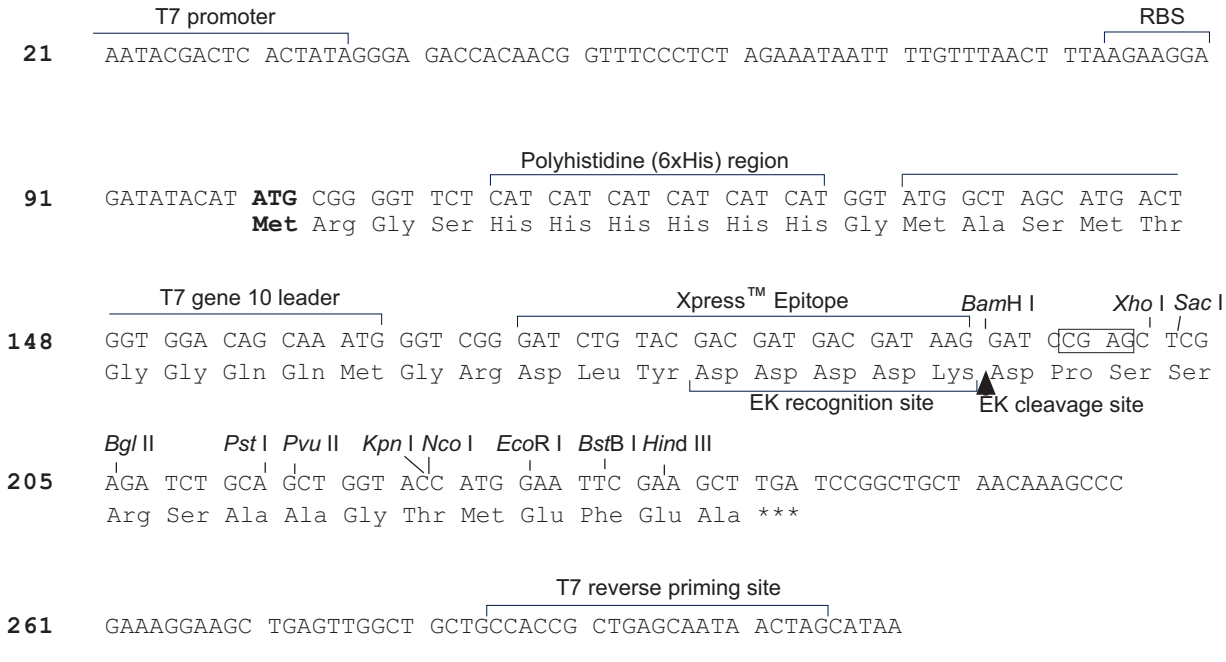
```
262  ACA AAG CCC GAA AGG AAG CTG AGT TGG CTG CTG CCA CCG CTG AGC AAT AAC TAG CAT
     Thr Lys Pro Glu Arg Lys Leu Ser Trp Leu Leu Pro Pro Leu Ser Asn Asn *** His
```

Continued on next page

Cloning into pRSET A, B, and C, continued

Multiple Cloning Site of pRSET B

Below is the multiple cloning site for pRSET B. Restriction sites are labeled to indicate the actual cleavage site. The boxed nucleotides indicate the variable region. Sequencing and functional testing have confirmed the multiple cloning site. The complete sequence of pRSET B is available for downloading at www.invitrogen.com or from Technical Support (see page 18). For a map and description of the features of pRSET B, please refer to pages 14–15.

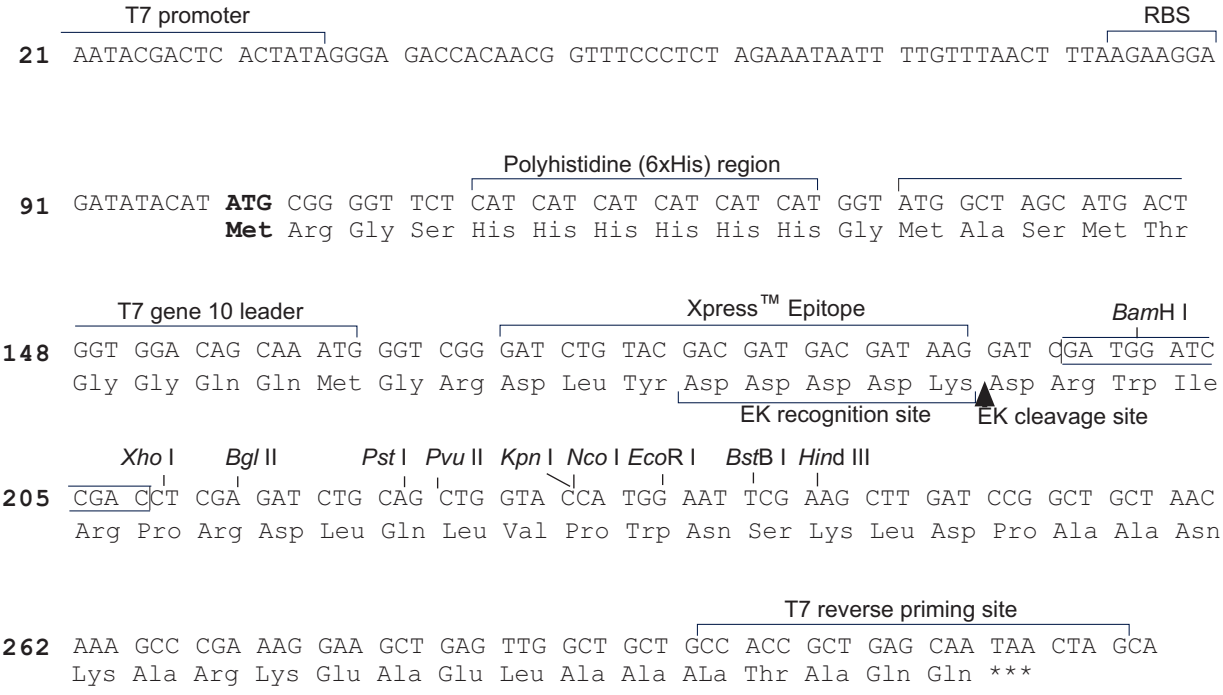


Continued on next page

Cloning into pRSET A, B, and C, continued

Multiple Cloning Site of pRSET C

Below is the multiple cloning site for pRSET C. Restriction sites are labeled to indicate the actual cleavage site. The boxed nucleotides indicate the variable region. Sequencing and functional testing have confirmed the multiple cloning site. The complete sequence of pRSET C is available for downloading at www.invitrogen.com or from Technical Support (see page 18). For a map and description of the features of pRSET C, please refer to pages 14–15.



Ligation

Once you have determined a cloning strategy, digest the appropriate version of pRSET with the selected restriction enzyme. Ligate your gene of interest into pRSETA, B, or C using standard molecular biology techniques.

Transformation

After ligating your gene of interest into the appropriate pRSET vector, transform the ligation mixture into competent TOP10F′. A detailed protocol for making competent TOP10F′ cells and using them for transformation is provided in the Appendix on page 17. Select 10–20 clones and analyze for the presence and orientation of your insert.

Continued on next page

Cloning into pRSET A, B, and C, continued

We recommend that you sequence your construct to confirm that your gene is in frame with the N-terminal tag and in the proper orientation. The T7 promoter primer (Cat. no. N560-02) is available for sequencing your insert in pRSET A, B, or C.

Making Frozen Glycerol Stocks

1. Grow 1–2 ml of the *E. coli* strain to be frozen in SOB medium overnight with antibiotic selection when appropriate.
2. Combine 0.85 ml of the overnight culture with 0.15 ml of sterile glycerol (sterilized by autoclaving).
3. Mix well by vortexing.
4. Transfer to an appropriate freezing vial (preferably a screw cap, air-tight gasket).
5. Freeze in an ethanol-dry ice bath or liquid nitrogen and then transfer to –80°C for long-term storage.

Expression

Introduction

BL21(DE3)pLysS cells are included with the kit as the host for expression. You will need pure plasmid DNA of your construct to transform into BL21(DE3)pLysS for expression studies. Since each recombinant protein has different characteristics that may affect optimal expression, it is helpful to do a pilot expression to determine the best conditions for optimal expression of your particular protein.

Preparation for Expression

To express your recombinant protein from pRSET, transform the plasmid into BL21(DE3)pLysS and select for ampicillin-resistant transformants (see page 17).

Before proceeding with the expression, streak out the BL21(DE3)pLysS transformant containing the recombinant plasmid on LB containing 35 µg/ml chloramphenicol and 50 µg/ml ampicillin. Chloramphenicol selects for maintenance of the pLysS plasmid required for T7 lysozyme expression and ampicillin selects for the pRSET plasmid (see **Appendix** for media recipes).

It is important to maintain BL21(DE3)pLysS strains on LB and chloramphenicol as loss of the plasmid will increase basal levels of transcription. We recommend preparing a frozen glycerol stock of untransformed BL21(DE3)pLysS (see page 7).

Plasmid Preparation

Plasmid DNA may be prepared using your method of choice. We recommend the S.N.A.P.™ MiniPrep Kit (Cat. no. K1900-01) or the PureLink™ HiPure Plasmid DNA Purification Kit (Cat. no. K2100-01) for isolation of pure plasmid DNA.

Positive Control Vector

Included in the kit is a stab of *E. coli* strain BL21(DE3)pLysS containing pRSET/*lac*Z. pRSET/*lac*Z is pRSET A with the β-galactosidase gene cloned into the *Bam*H I and *Hind* III sites for use as a positive control for expression. β-galactosidase should appear as a band of approximately 120 kDa on a denaturing polyacrylamide gel. The complete sequence of this vector is available at www.invitrogen.com or from Technical Support (page 18).

Continued on next page

Expression, continued

Pilot Expression

Expression conditions will vary depending on the nature of your protein; therefore, we recommend performing a time course experiment to optimize expression of your recombinant protein.

1. Inoculate 2 ml of SOB containing ampicillin (50 µg/ml) and chloramphenicol (35 µg/ml) with a single recombinant *E. coli* colony. Grow overnight at 37°C with shaking.
2. The next day, inoculate 25 ml of SOB (it is not necessary to include antibiotics for expression) to an OD_{600} of 0.1 with the overnight culture.
3. Grow the culture at 37°C with vigorous shaking to an OD_{600} = 0.4–0.6.
4. Remove a 1 ml aliquot of cells prior to IPTG induction, centrifuge the sample in a microcentrifuge, and aspirate the supernatant. Freeze the cell pellet at –20°C. This will be the time zero sample.
5. Add IPTG to a final concentration of 1 mM (0.25 ml of 100 mM IPTG stock to 25 ml culture) and continue to grow the cells. See page 12 for preparation of the IPTG stock solution.
6. After 1 hour of incubation, remove a 1 ml sample, centrifuge as described in Step 4, aspirate the supernatant, and freeze the cell pellet at –20°C. Continue to take samples at 1 hour intervals for 4 to 6 hours.
7. When all time points have been collected, resuspend each pellet in 100 µl of 20 mM phosphate buffer at neutral pH, and freeze in liquid nitrogen or methanol/dry ice (exercise caution when handling liquid nitrogen, it can cause severe burns if it comes in contact with the skin, wear appropriate protective equipment). Thaw the frozen lysate at 42°C.
8. Repeat this freeze-thaw two to three additional times and pellet the insoluble protein in a microcentrifuge for 10 minutes at maximum speed at 4°C.
9. Remove the supernatant to a fresh labeled tube. To 100 µl of supernatant sample, add an equal volume of 2X SDS-PAGE sample buffer. Resuspend the pellet in 100 µl of 1X SDS-PAGE sample buffer.
10. Load 10–20 µl of each of the supernatant and pellet samples after boiling for 5 minutes on an appropriate SDS-PAGE gel and electrophorese.

Analysis of Samples

1. Stain the gel with Coomassie blue and look for a band of increasing intensity in the expected size range for the recombinant protein. Use the uninduced culture as a negative control. From this expression experiment, determine the optimal time after IPTG induction to harvest the cells.
2. In addition, you may perform a western blot to confirm that the overexpressed band is your desired protein (see next page).
3. Use the positive control to confirm that growth and induction were performed properly. The pRSET/*lac*Z vector should produce an ~120 kDa protein when induced with IPTG.

Note

Expression of your protein with the N-terminal tag will increase the size of your protein by approximately 3 kDa. Be sure to account for any additional amino acids between the tag and your protein.

Continued on next page

Expression, continued

Detecting Recombinant Fusion Proteins

To detect expression of your recombinant fusion protein by western blot analysis, you may use antibodies against the appropriate epitope available from Invitrogen (see page v for ordering information) or an antibody to your protein of interest. In addition, the Positope™ Control Protein (Cat. no. R900-50) is available from Invitrogen for use as a positive control for detection of fusion proteins containing an Xpress™ or HisG epitope. The ready-to-use WesternBreeze® Chromogenic Kits and WesternBreeze® Chemiluminescent Kits are available from Invitrogen to facilitate detection of antibodies by colorimetric or chemiluminescent methods. For more information, please refer to our website (www.invitrogen.com) or call Technical Support (see page 18).

Expressing Recombinant Protein

1. Inoculate 2 ml of SOB containing ampicillin (50 µg/ml) and chloramphenicol (35 µg/ml) with a single recombinant *E. coli* colony. Grow overnight at 37°C with shaking (225 rpm).
2. The next day, inoculate 25 ml of SOB to an OD_{600} of 0.1 with the overnight culture. Antibiotics are not required for expression. Please note that you may increase the volume to produce more protein.
3. Grow the culture at 37°C with shaking (225 rpm) to an OD_{600} = 0.4–0.6.
4. Add IPTG to a final concentration of 1 mM (0.25 ml of 100 mM IPTG stock to 25 ml culture).
5. Grow the culture at 37°C with vigorous shaking for the optimal time determined in pilot expression (see page 9).
6. Harvest the cells by centrifugation and either proceed directly to lysis or freeze the cells at –80°C until ready for use.

Troubleshooting Expression

Use the information provided in the table below to troubleshoot your expression experiment.

Problem	Probable Cause	Possible Solution
No or low expression	Insert ligated into wrong reading frame	Check sequence carefully and determine which vector, pRSET A, B, or C is appropriate with the restriction site selected
	Kinetics of induction different than expected	Try a longer time course for induction than the 4–5 hours recommended
	Not induced at OD_{600} 0.4–0.6	Induce expression at OD_{600} 0.4–0.6
	IPTG solution is too old	Prepare a fresh solution of IPTG or use up to 10 mM IPTG
	Protein is difficult to detect on a Coomassie-stained gel	Perform a western blot using the Anti-Xpress™ antibody for detection

Purification

Introduction

Once you have expressed your recombinant fusion protein, you may purify your fusion protein using a metal-chelating resin such as ProBond™ (available from Invitrogen, Cat. no. R801-01).

ProBond™

ProBond™ is a nickel-charged Sepharose® resin that can be used for affinity purification of fusion proteins containing the 6×His tag. Proteins bound to the resin may be eluted with either low pH buffer or competition with imidazole or histidine.

- To scale up your pilot expression for purification, see below.
- To purify your fusion protein using ProBond™, refer to the ProBond™ Purification System manual for instruction. The ProBond™ Purification System manual is available for downloading at www.invitrogen.com.

To purify your fusion protein using another metal-chelating resin, refer to the manufacturer's instructions.

Binding Capacity of ProBond™

One milliliter of ProBond™ binds at least 1 mg of recombinant protein. This amount can vary depending on the nature of the protein.

Scale-up of Expression for Purification on ProBond™

Please note that the capacity of ProBond™ is about 1 mg of protein per milliliter. Depending on the expression level of your recombinant fusion protein, you may need to adjust the culture volume to bind the maximum amount of recombinant fusion protein to your column. For a prepacked 2 ml ProBond™ column, start with 50 ml of bacterial culture.

If you need to purify larger amounts of recombinant protein, you may need more ProBond™ resin. See page v for ordering information.

To grow and induce a 50 ml bacterial culture:

1. Inoculate 10 ml of SOB or LB containing 50–100 µg/ml ampicillin and 34 µg/ml chloramphenicol (if needed) with a single recombinant *E. coli* colony.
2. Grow overnight at 37°C with shaking (225–250 rpm) to OD_{600} = 1–2.
3. The next day, inoculate 50 ml of SOB or LB containing 50-100 µg/ml ampicillin with 1 ml of the overnight culture. **Note**: You can scale up further and inoculate all of the 10 ml overnight culture into 500 ml of medium, but you may need a larger bed volume for your ProBond™ column.
4. Grow the culture at 37°C with shaking (225–250 rpm) to an OD_{600} = ~0.5 (2–3 hours). The cells should be in mid-log phase.
5. Add 1 mM IPTG to induce expression.
6. Grow at 37°C with shaking until the optimal time point determined by the pilot expression is reached. Harvest the cells by centrifugation (3000 × g for 10 minutes at 4°C).
7. At this point, you may proceed directly to purification, or store the cells for future use at –80°C.

Appendix

Recipes

SOB (For 1 Liter)

To 950 ml of deionized water add:

20.0 g Tryptone
5.0 g Yeast Extract
0.5 g NaCl
186.0 mg KCl

1. Mix the solution until dissolved.
2. Adjust the pH to 7.0 with 5 N NaOH (approximately 0.2 ml).
3. If making solid media (for plates or top agar), add 15 g of agar after adjusting the pH.
4. Adjust the volume to 1000 ml and sterilize by autoclaving.
5. Once autoclaved, add 10 ml of sterile 1 M Mg^{2+} (e.g. 10 ml of sterile 1 M $MgCl_2$ or sterile 1 M $MgSO_4$).

SOC (For 1 Liter)

Follow recipe as per SOB. After autoclaving, let cool to about 60°C and add 10 ml of 50% glucose. Mix the media well.

LB (For 1 Liter)

Component	liquid	plates	top agar
Tryptone	10 g	10 g	10 g
Yeast Extract	5 g	5 g	5 g
NaCl	10 g	10 g	10 g
Agar	-	15 g	7 g

1. Combine the tryptone, yeast extract, and NaCl with 950 ml of deionized water. Mix the solution until dissolved.
2. Adjust the pH to 7.0 with 5 N NaOH (will take about 0.2 ml). If making solid media (for plates or top agar) add the appropriate amount of agar after adjusting the pH.
3. Adjust volume to 1 liter with water.
4. Sterilize by autoclaving.
5. After autoclaving add antibiotic, if desired. Add chloramphenicol to a final concentration of 10 µg/ml and ampicillin to a final concentration of 50 µg/ml.

Continued on next page

Recipes, continued

Antibiotics

Ampicillin:

Prepare a stock solution of 50 mg/ml in deionized water and filter sterilize it with a 0.22 µm filter. To prepare selective medium, cool medium to ~50°C after autoclaving, and add 1 ml of the ampicillin stock per liter of media (both liquid and solid) for a final concentration of 50 µg/ml. Store the stock solution at –20°C.

Chloramphenicol:

Prepare a stock solution of 35 mg/ml in 100% ethanol. It is not necessary to filter-sterilize. Store the stock solution at –20°C. To prepare selective medium, cool the medium to ~50°C after autoclaving and add 1 ml of the stock solution per liter of medium for a final concentration of 35 µg/ml.

100 mM IPTG

For 10 ml of a 100 mM solution:

Dissolve 0.24 g of IPTG in sterile, deionized water. Bring the final volume to 10 m and filter sterilize (0.22 µm filter). Do not autoclave.

50 mM $CaCl_2$

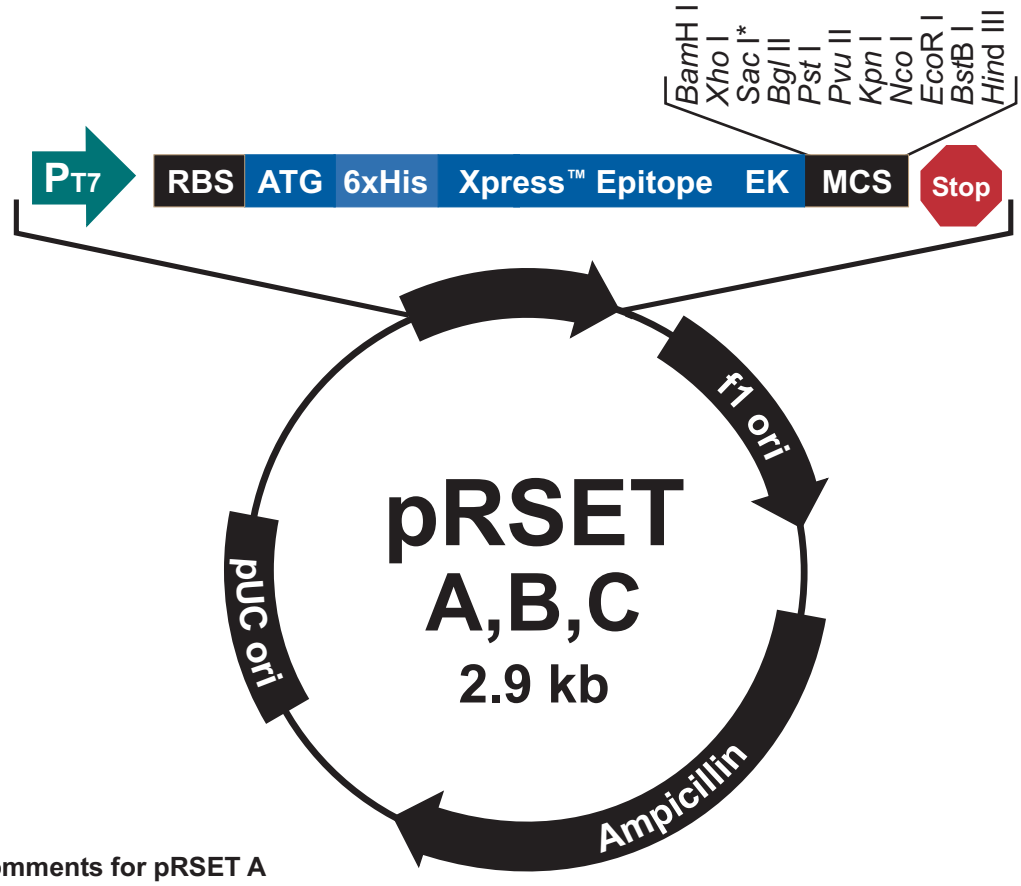
For 100 ml of a 50 mM solution:

Dissolve 0.56 g of anhydrous $CaCl_2$ (MW = 111) in 100 ml of deionized water. Filter sterilize (0.22 µm filter) or autoclave. Use this solution ice cold for competent cell preparation.

Map of pRSET A, B, and C

pRSET A, B, and C

The map below shows the features of pRSET A, B, and C. **The complete sequence of the vector is available for downloading from our website at www.invitrogen.com or from Technical Support (see page 18).**



*Version C does not contain *Sac* I

Comments for pRSET A
2897 nucleotides

T7 promoter: bases 20-39
6xHis tag: bases 112-129
T7 gene 10 leader: bases 133-162
Xpress™ epitope: bases 169-192
Multiple cloning site: bases 202-248
T7 reverse priming site: bases 295-314
T7 transcription terminator: bases 256-385
f1 origin: bases 456-911
bla promoter: bases 943-1047
Ampicillin (*bla*) resistance gene (ORF): bases 1042-1902
pUC origin: bases 2047-2720 (C)

Features of pRSET A, B, and C

Features

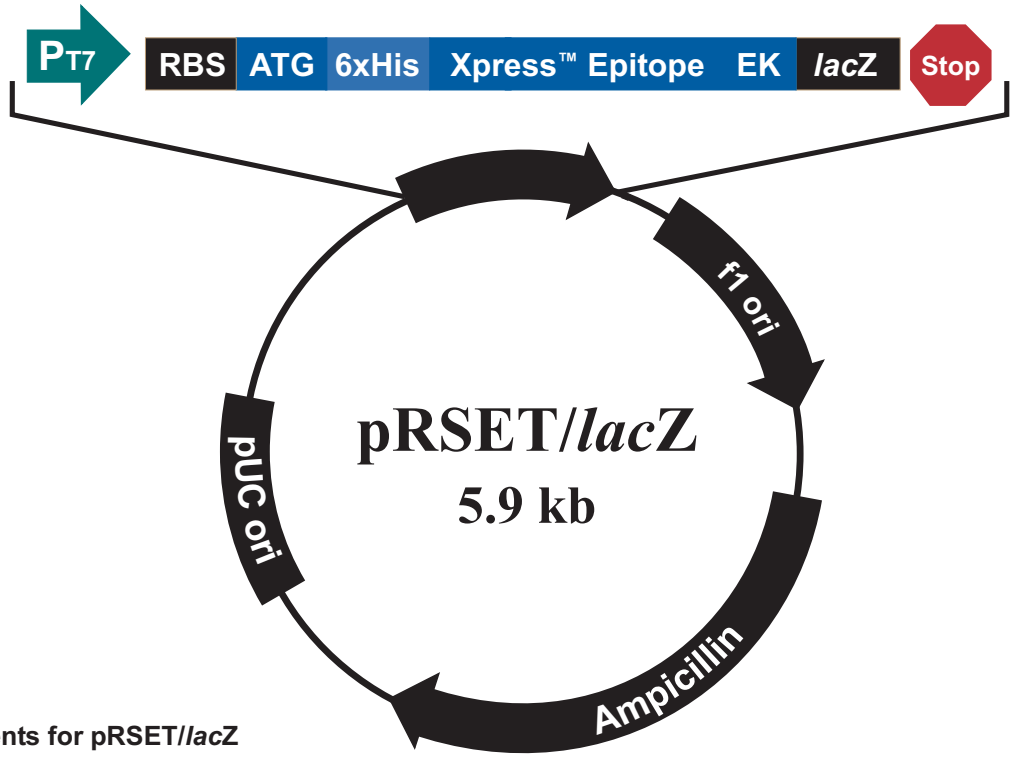
The important elements of pRSET A, B, and C are described in the table below. All features have been functionally tested.

Feature	Benefit
T7 promoter	Provides tight, dose-dependent regulation of heterologous gene expression. Provides a binding site for most T7 promoter primers for sequencing into the insert.
Ribosome binding site	Optimally spaced from the multiple cloning site for efficient translation of the gene of interest.
Initiation ATG	Provides a translational initiation site for the fusion protein.
N-terminal 6×His tag	Permits purification of recombinant fusion protein on metal-chelating resins (i.e. ProBond™). In addition, it allows detection of the recombinant protein with the Anti-HisG Antibody (R940-25) or the Anti-HisG-HRP Antibody (Cat. no. R941-25)
T7 gene 10 sequence	Provides protein stability
N-terminal Xpress™ epitope tag	Allows detection of the fusion protein by the Xpress™ Antibody (Cat. no. R910-25) or the Xpress™-HRP Antibody (Cat. no. R911-25)
Enterokinase cleavage site	Provides a site for efficient removal of the fusion tag.
Multiple cloning site	Allows insertion of your gene of interest and facilitates in cloning in frame with the N-terminal epitope tag.
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 resistance gene (β-lactamase)	Allows selection of the plasmid in *E. coli*.
pUC origin	High copy replication and growth in *E. coli*.

Map of pRSET/*lacZ*

Description

pRSET/*lacZ* is a 5911 bp control vector expressing β-galactosidase. Note that -galactosidase is fused to an N-terminal peptide containing the Xpress™ peptide, 6xHis tag and an enterokinase recognition site. The molecular weight is approximately 120 kDa. The figure below summarizes the features of the pRSET/*lacZ* vector. **The complete sequence of the vector is available for downloading from our website at www.invitrogen.com or from Technical Support (see page 18).**



Comments for pRSET/*lacZ*
5911 nucleotides

T7 promoter: bases 20-39
6xHis tag: bases 112-129
T7 gene 10 leader: bases 133-162
Xpress™ epitope: bases 169-192
lacZ ORF: bases 199-3258
T7 reverse priming site: bases 295-314
T7 transcription terminator: bases 3270-3399
f1 origin: bases 3470-3925
bla promoter: bases 3957-4061
Ampicillin (*bla*) resistance gene (ORF): bases 4056-4916
pUC origin: bases 3930-5866 (C)

Transformation Protocol for TOP10F′ and BL21(DE3)pLysS

Introduction

This protocol is provided for your convenience. Other protocols may be suitable. Use the table below to select the appropriate medium for use with TOP10F′ or BL21(DE3)pLysS.

Strain	Maintenance Medium	pRSET Selection Medium
TOP10F′	LB + 10 µg/ml tetracycline	LB + 50 µg/ml ampicillin
BL21(DE3)pLysS	LB + 35 µg/ml chloramphenicol	LB + 50 µg/ml ampicillin + 35 µg/ml chloramphenicol

Protocol

1. Take the desired stab and streak out a small portion on the appropriate maintenance medium and incubate at 37°C overnight. The stab should remain viable for several months when stored at 4°C in the dark. We recommend making a frozen glycerol stock for long-term storage (see page 7).
2. Pick a single colony and transfer it into 100 ml of SOB medium in a 1 liter flask (see page 12 for media recipes). Incubate the flask at 37°C with vigorous shaking (> 200 cycles/minute in a rotary shaker).
3. When the OD_{600} reaches approximately 0.5, collect the cells by centrifuging at 4000 rpm for 10 minutes in a 4°C rotor (Sorvall GSA).
4. Resuspend the pellet in 10 ml of ice-cold 50 mM $CaCl_2$. Keep the cells on ice for at least 30 minutes.
5. Centrifuge the $CaCl_2$-treated cells in a 4°C rotor (Sorvall SS-34) at 4000 rpm for 5 minutes. Gently resuspend the cells in 4 ml of ice-cold 50 mM $CaCl_2$. Keep the cells on ice.
6. Aliquot 100 µl of the $CaCl_2$-treated cells for each transformation into a prechilled microcentrifuge tube. Store the cells at –80°C for long-term storage.
7. For transformation, take one tube of 100 µl of competent cells (prepared above) and add the plasmid DNA (10–100 ng) to the cells. Incubate the cells on ice for 30 minutes.
8. Heat shock cells at 42°C for 45 seconds (in a water bath). Return the tube(s) to ice for 2 minutes.
9. Add 1 ml of SOC media and incubate the culture(s) for 45 minutes at 37°C with vigorous shaking (> 200 cycles/minute in a rotary shaker).
10. Plate the appropriate amount of cells onto SOB plates containing the appropriate antibiotic selection for the plasmid (for pRSET vectors use ampicillin).
    Note: When selecting for transformants in BL21(DE3)pLysS, include 35 µg/ml chloramphenicol in the plate.

For your convenience, One Shot® TOP10F′ or BL21(DE3)pLysS competent cells are available for high efficiency transformation. See page v for more information.

Technical Support

Web Resources

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

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

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

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SDS

Safety Data Sheets (SDSs) are available at www.invitrogen.com/sds.

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Reference

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994). Current Protocols in Molecular Biology (New York: Greene Publishing Associates and Wiley-Interscience).

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