invitrogen™

pTrcHis2 A, B, and C

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User Manual

Table of Contents

Kit Contents and Storage

Shipping and Storage

pTrcHis2 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.**

Note: For long-term storage of your stab, we recommend preparing a glycerol stock immediately upon receipt and storing at –80°C.

Vector	Composition	Amount
pTrcHis2 A, B, and C	40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	20 µg
pTrcHis2/*lacZ*	40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	20 µg
TOP10 *E. coli* stab	-	1 stab

Introduction

Product Overview

pTrcHis2

The pTrcHis2 plasmids are pBR322-derived expression vectors designed for efficient recombinant protein expression and purification in *E. coli*. High levels of expression are possible using the *trc* (*trp-lac*) promoter (Egon *et al.*, 1983) and the *rrn*B anti-termination region (Li *et al.*, 1984). The *trc* promoter contains the –35 region of the *trp* promoter together with the –10 region of the *lac* promoter (Brosius *et al.*, 1985; Egon *et al.*, 1983; Mulligan *et al.*, 1985). To regulate expression, the gene encoding Lac repressor (*lac*I^q) is provided in the pTrcHis2 vectors, allowing regulation of the *trc* promoter regardless of whether the host strain contains a gene encoding the Lac repressor.

Isopropyl-β-D-thiogalactopyranoside (IPTG) is used to induce expression of your gene. Translation is enhanced by the bacteriophage T7 gene 10 translation enhancer and a minicistron that provides highly efficient translational restart into the open reading frame of the multiple cloning site. DNA inserts are positioned downstream and in frame with the initiation ATG and a C-terminal fusion peptide. The C-terminal peptide encodes the *myc* epitope and six histidine residues that function as a metal binding site in the expressed protein.

Methods

Cloning into pTrcHis2

General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, DNA sequencing, and DNA biochemistry, see *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

Maintaining pTrcHis2

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 TOP10, DH5α, or equivalent. Transformants are selected on LB plates containing 50–100 µg/mL ampicillin.

E. coli Strain

TOP10 is provided for growth and maintenance of these plasmids. This strain is provided as a convenience for those who do not have access to other *E. coli* strains. Many *E. coli* strains are suitable for the growth of this vector. We recommend that you propagate vectors containing inserts in recombination deficient (*rec*A), endonuclease A-deficient (*end*A) *E. coli* strains.

Genotype: F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G

TOP10 contains:

- *rec*A for stable replication of high copy number plasmids
- *end*A for improved yield and quality of miniprep DNA
- *hsd*RMS to eliminate cleavage of recombinant plasmid by the endogenous *Eco*R restriction system

For your convenience, TOP10 is available as competent cells from Invitrogen (see page 12).

Cloning in the pTrcHis2 Vectors

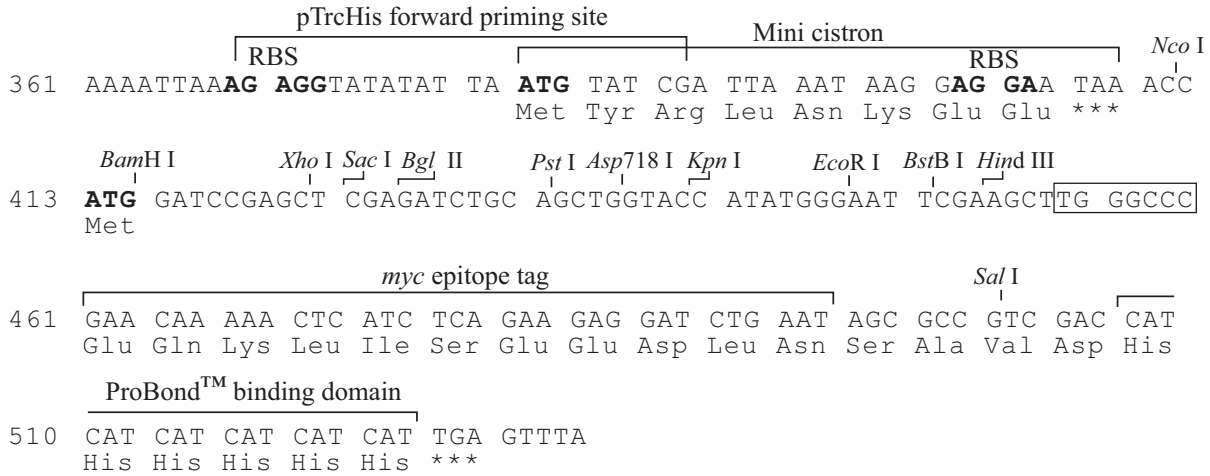
To generate recombinant proteins that are expressed correctly and contain the C-terminal fusion peptide, it is necessary to clone in frame with **BOTH** the initiation ATG (bp 413–415) and the C-terminal peptide. To facilitate cloning, the pTrcHis2 vector is provided in three different reading frames. They differ only in the spacing between the sequences that code for the multiple cloning site and the C-terminal peptide. For proper expression, first determine which restriction sites are appropriate for ligation and then which vector will preserve the reading frame at **BOTH** the 5´ and the 3´ ends. You may have to use PCR to create a fragment with the appropriate restriction sites to clone in frame at both ends. Be sure that there is no stop codon in the open reading frame of your gene.

Continued on next page

Cloning into pTrcHis2, Continued

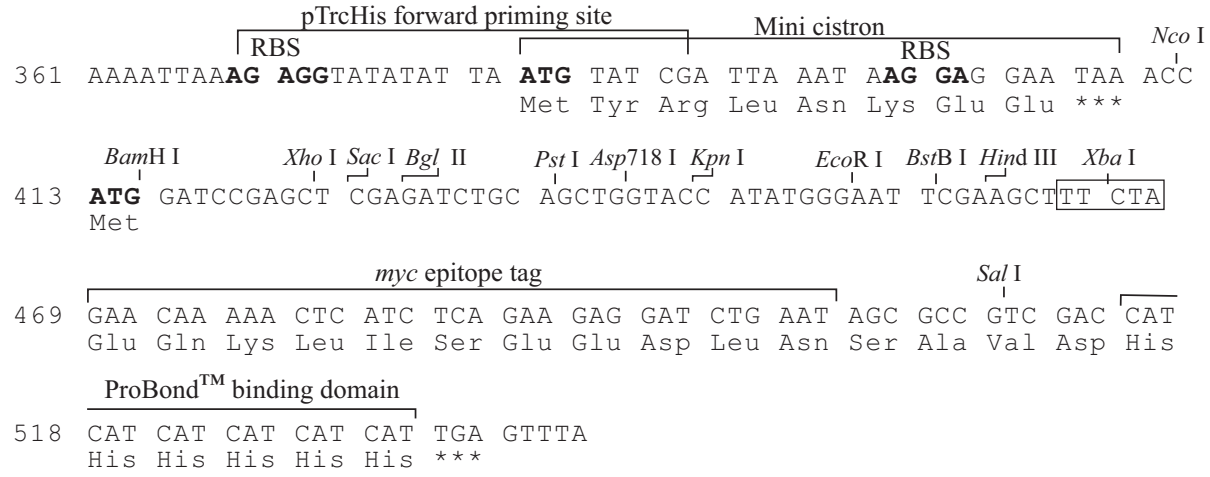
Multiple Cloning Site of pTrcHis2 A

Below is the multiple cloning site for pTrcHis2 A. Restriction sites are labeled to indicate cleavage site. The boxed sequence is the variable region that facilitates in frame cloning with the C-terminal peptide. This variable region is located between the *Hin*d III site and the *myc* epitope. The multiple cloning site has been confirmed by sequencing and functional testing.



Multiple Cloning Site of pTrcHis2 B

Below is the multiple cloning site for pTrcHis2 B. Restriction sites are labeled to indicate cleavage site. The boxed sequence is the variable region that facilitates in frame cloning with the C-terminal peptide. This variable region is located between the *Hin*d III site and the *myc* epitope. The multiple cloning site has been confirmed by sequencing and functional testing.



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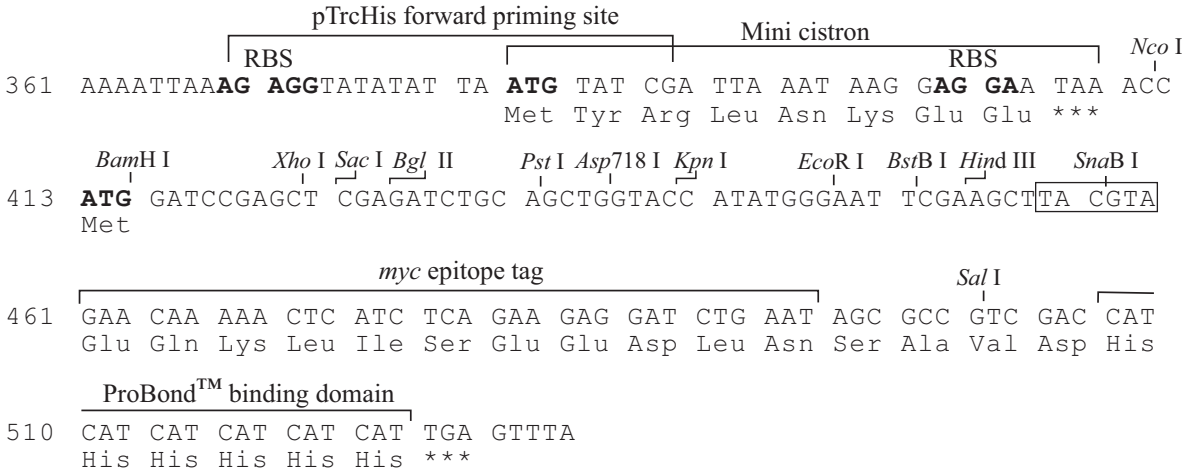
Cloning into pTrcHis2, Continued

Multiple Cloning Site of pTrcHis2 C

Below is the multiple cloning site for pTrcHis2 C. Restriction sites are labeled to indicate cleavage site. The boxed sequence is the variable region that facilitates in frame cloning with the C-terminal peptide. This variable region is located between the *Hin*d III site and the *myc* epitope. The multiple cloning site has been confirmed by sequencing and functional testing.



E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g., TOP10, DH5α) and select on LB plates containing 50–100 µg/mL ampicillin. Select 10–20 clones and analyze for the presence and orientation of your insert.

We recommend that you sequence your construct with the pTrcHis Forward primer to confirm that your gene is in frame with the initiation ATG and the C-terminal peptide. For ordering primers, see page 11.

Preparing a Glycerol Stock

Once you have obtained your construct, we recommend that you store your clone as a glycerol stock.

1. Grow 1 to 2 mL of the strain containing your construct in pTrcHis2 to saturation.
2. Combine 0.85 mL of the stationary culture with 0.15 mL of sterile glycerol.
3. Mix the solution by vortexing.
4. Transfer to an appropriate vial for freezing and cap.
5. Freeze in an ethanol/dry ice bath or liquid nitrogen and then transfer to -80°C for long-term storage.

Expression

Introduction

Since each recombinant protein has different characteristics that may affect optimum expression, it is helpful to run a time course of expression to determine the optimal time for maximum expression of your particular protein. A mock expression consisting of the pTrcHis2 vector alone should be done as a negative control. pTrcHis2/*lacZ* is provided for use as a positive expression control (see page 9). Transform all plasmids into TOP10 *E. coli* (or similar strains) to analyze expression (see page 11).

Materials Needed

- SOB or LB containing 50 µg/mL ampicillin (see **Recipes**, page 10)
- 37°C shaking incubator
- 100 mM IPTG
- 1X and 2X SDS-PAGE sample buffer
- Reagents and apparatus for SDS-PAGE gel

Pilot Expression

1. For each strain, inoculate 2 mL of SOB or LB containing 50 µg/mL ampicillin with a single recombinant *E. coli* colony.
2. Grow overnight at 37°C with shaking (225–250 rpm).
3. The next day, inoculate 10 mL of SOB or LB containing 50 µg/mL ampicillin with 0.2 mL of the overnight culture.
4. Grow the culture at 37°C with vigorous shaking to an OD600 = 0.6 (the cells should be in mid-log phase).
5. Remove a 1 mL aliquot of cells, centrifuge at maximum speed in a microcentrifuge for 30 seconds, and aspirate the supernatant.
6. Freeze the cell pellet at –20°C. This is the zero time point sample.
7. Add IPTG to a final concentration of 1 mM (0.1 mL of a 100 mM IPTG stock to 10 mL) and grow at 37°C with shaking.
8. Take 1 mL samples every hour for 5 hours (or more) and treat as in Steps 5 and 6. Label each tube to correspond to the number of hours postinduction.

Preparing Time Point Samples

1. When all the time points have been collected, resuspend each pellet in 100 µL of 1X SDS-PAGE sample buffer.
2. Boil 5 minutes and centrifuge briefly.
3. Analyze 5 µL of each sample on an appropriate SDS-PAGE gel.

Continued on next page

Analysis of Time Point 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.

   Note: The *myc* epitope and polyhistidine region contribute 2.5 kDa to your protein. Be sure and account for any additional amino acids at the N-terminus and between the 3´ cloning site and the *myc* epitope.
2. Use the negative control to distinguish recombinant proteins from background proteins.
3. Use the positive control to confirm that growth and induction was done properly. The positive control should yield a 120 kDa protein with maximum expression occurring between 3–4 hours.
4. You should be able to determine the optimal time point for maximum expression.

Expression of Recombinant Protein

Use the conditions determined above to grow and induce 50 mL of cells. This is the largest culture volume to use with the 2 mL prepacked columns included in the ProBond™ Purification System. If you need to purify larger amounts of recombinant protein, you may need more ProBond™ resin. See page 11 for ordering information.

1. Inoculate 2 mL of SOB or LB containing 50 µg/mL ampicillin with a single recombinant *E. coli* colony.
2. Grow overnight at 37°C with shaking (225–250 rpm).
3. The next day, inoculate 50 mL of SOB or LB containing 50 µg/mL ampicillin with 1 mL of the overnight culture.
4. Grow the culture at 37°C with vigorous shaking to an OD600 = 0.6 (the cells should be in mid-log phase).
5. Add IPTG to a final concentration of 1 mM (0.5 mL of a 100 mM IPTG stock to 50 mL).
6. Grow at 37°C with shaking until the optimal time point is reached. Harvest the cells by centrifugation (3,000 × g for 10 minutes at 4°C).
7. At this point, you may proceed directly to purification (ProBond™ Purification System manual) or store at –80°C for future use.

Detection and Purification of Recombinant Proteins

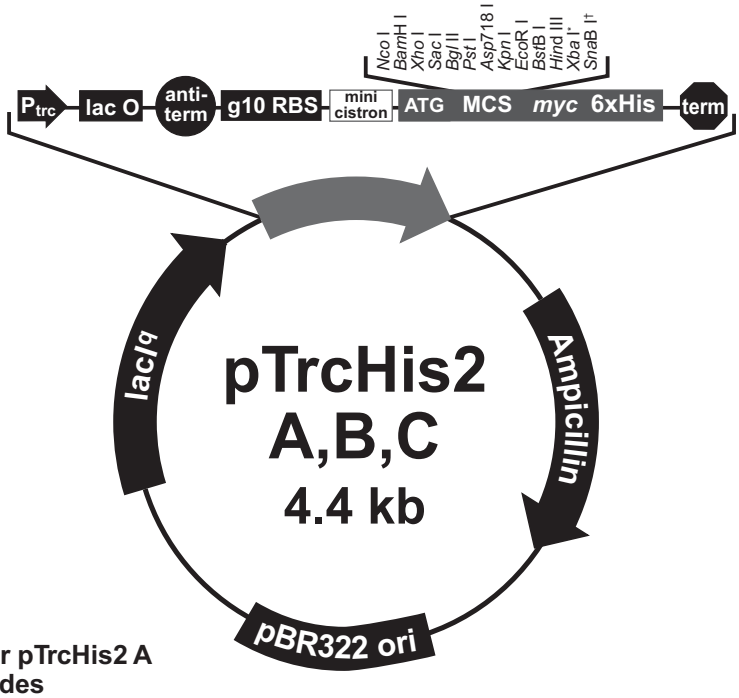
Expression of your recombinant protein can be detected using an antibody to the *myc* epitope encoded in the C-terminal fusion peptide. In addition, the metal binding domain allows simple, one-step purification of your recombinant protein by Immobilized Metal Affinity Chromatography (IMAC) using Invitrogen's ProBond™ Resin (for ordering, see page 11).

Appendix

pTrcHis2 Vectors

Map of pTrcHis2

The figure below summarizes the features of the pTrcHis2 vectors. The sequences for all three pTrcHis2 vectors can be downloaded from our website (www.invitrogen.com) or by contacting **Technical Support** (see page 12). Details of each multiple cloning site are shown on pages 3–4.



Comments for pTrcHis2 A
4406 nucleotides

* *Xba* I is only found in pTrcHis2 B

† *Sna*B I is only found in pTrcHis2 C

trc promotor region: bases 190-382
-35 region: bases 193-198
-10 region: bases 216-221
lac operator (*lac*O): bases 228-248
*rrn*B antitermination signal: bases 264-333
gene 10 region: bases 346-354
Ribosome binding site: bases 369-373
pTrcHis forward priming site: bases 370-390
Minicistron ORF: bases 383-409
Reinitiation RBS: bases 398-403
Expression ATG: bases 413-415
Multiple cloning site: bases 411-464
myc epitope: bases 471-503
Polyhistidine tag: bases 516-533
*myc*His reverse priming site: bases 508-527
*rrn*B T1 and T2 transcriptional terminators: bases 639-796
Ampicillin resistance ORF: bases 1076-1936
pBR322 origin: bases 2081-2754
Lac Repressor (*lacI*^{q}) ORF: bases 3408-4367

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Features of pTrcHis2

The important elements of pTrcHis2 A (4406 bp), pTrcHis2 B (4404 bp), and pTrcHis2 C (4405 bp) are described in the following table. All features have been functionally tested.

Feature	Benefit
trc promoter	–35 (*trp*B) and –10 (*lac*UV5) hybrid promoter for high-level expression of fusion protein (Brosius *et al.*, 1985; Egon *et al.*, 1983; Mulligan *et al.*, 1985).
lac operator (*lac*O)	Permits binding of the Lac repressor to repress transcription.
*rrn*B antitermination region	Reduces the level of premature transcription termination(Li *et al.*, 1984).
Bacteriophage gene 10 translational enhancer	Optimizes translation initiation of minicistron (Olins *et al.*, 1988).
Minicistron and reinitiation ribosome binding site	Contains a second ribosome site for efficient reinitiation of translation into the gene of interest (Schoner *et al.*, 1986).
Initiation ATG	Provides a translation initiation site for the fusion protein.
Multiple cloning site	Allows insertion of your gene for expression.
C-terminal *myc* epitope tag (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu-Asn)	Allows detection of the fusion protein by the Anti-*myc* Antibody (Evan *et al.*, 1985) (for ordering, see page 11).
C-terminal polyhistidine region	Formation of the metal-binding site for affinity purification of recombinant protein.
Ampicillin resistance gene (β-lactamase)	Allows selection of the plasmid in *E. coli.*
pBR322 origin	Low copy replication and growth in *E. coli.*
*lac*I^{q} gene	Encodes and overproduces the Lac repressor protein.

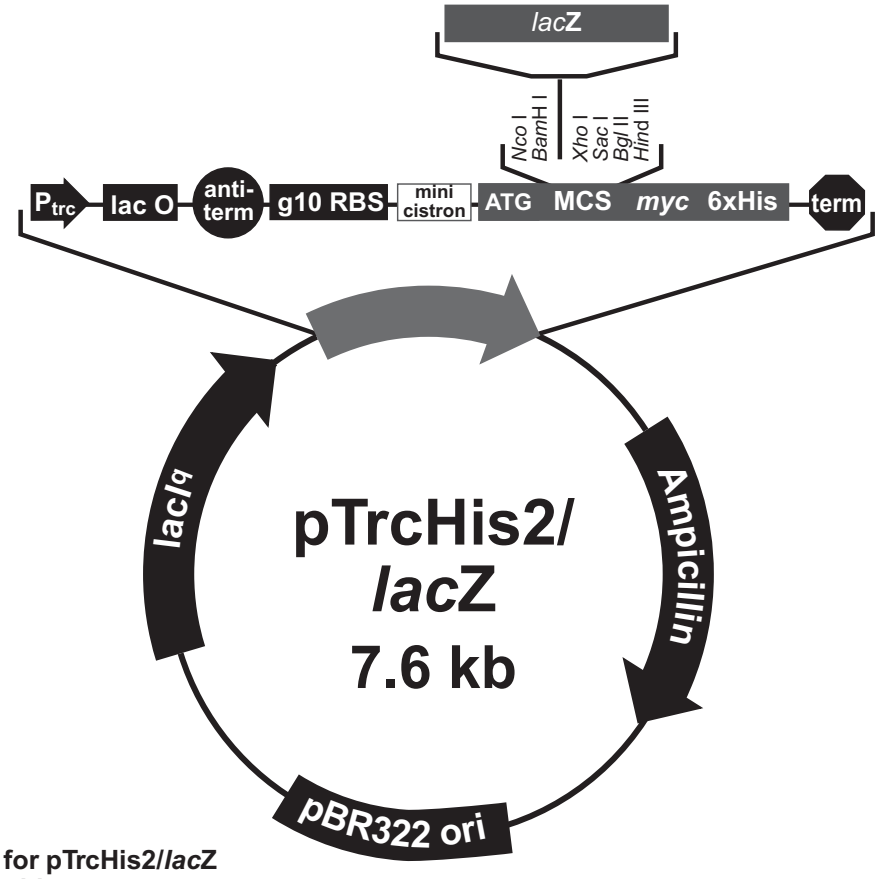
pTrcHis2/*lacZ*

Description

pTrcHis2/*lacZ* is a 7552 bp control vector containing the gene for β-galactosidase. It was constructed by digesting pTrcHis2 A with *Bam*H I and *Xho* I. A 3.2 kb *Bam*H I-*Xho* I fragment containing the *lacZ* gene was then ligated into pTrcHis2 A. The vector expresses a 120 kDa protein.

Map of Control Vector

The figure below summarizes the features of the pTrcHis2/*lacZ* vector. The nucleotide sequence for pTrcHis2/*lacZ* may be downloaded from our website (www.invitrogen.com) or by contacting **Technical Support** (see page 12).



Comments for pTrcHis2/*lacZ*
7552 nucleotides

trc promotor region: bases 190-382
-35 region: bases 193-198
-10 region: bases 216-221
lac operator (*lac*O): bases 228-248
*rrn*B antitermination region: bases 264-333
gene 10 region: bases 346-354
Ribosome binding site: bases 369-373
Minicistron ORF: bases 383-409
Reinitiation RBS: bases 398-403
Expression ATG: bases 413-415
*lac*Z ORF: bases 467-3523
myc epitope: bases 3617-3649
Polyhistidine tag: bases 3662-3679
*myc*His reverse priming site: bases 3654-3673
Ampicillin resistance ORF: bases 4222-5082
pBR322 origin: bases 5227-5900
Lac Repressor (*lac*I^{q}) ORF: bases 6554-7513

Recipes

LB Medium (with Ampicillin)

LB Medium (per liter)

1% Tryptone
0.5% Yeast Extract
0.5% NaCl
pH 7.0

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 5 g NaCl in 950 mL deionized water.
2. Adjust the pH of the solution to 7.5 with 5 M NaOH and bring the volume to 1 liter.
3. Autoclave for 20 minutes on liquid cycle.
4. Let solution cool to ~55°C. Add ampicillin to a final concentration of 50 µg/mL.
5. Store the medium at 4°C. Medium is stable for only 1–2 weeks.

LB Agar Plates with Ampicillin

LB Medium (per liter)

1% Tryptone
0.5% Yeast Extract
0.5% NaCl
1.5% Agar
pH 7.0

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 5 g NaCl in 950 mL deionized water.
2. Adjust the pH of the solution to 7.5 with 5 M NaOH, add 15 g agar, and bring the volume to 1 liter.
3. Autoclave for 20 minutes on liquid cycle.
4. Let agar cool to ~55°C. Add ampicillin to a final concentration of 50 µg/mL.
5. Pour into 10 cm petri plates. Let the plates harden, then invert and store at 4°C. Plates containing ampicillin are stable for 1–2 weeks.

SOB Medium (with Ampicillin)

SOB (per liter)
2% Tryptone
0.5% Yeast Extract
0.05% NaCl
2.5 mM KCl
10 mM $MgCl_2$

1. Dissolve 20 g tryptone, 5 g yeast extract, and 0.5 g NaCl in 950 mL deionized water.
2. Make a 250 mM KCl solution by dissolving 1.86 g of KCl in 100 mL of deionized water. Add 10 mL of this stock KCl solution to the solution in Step 1.
3. Adjust pH to 7.5 with 5 M NaOH and add deionized water to 1 liter.
4. Autoclave this solution, cool to ~55°C, and add 10 mL of sterile 1 M MgCl2. You may also add ampicillin to 50 µg/mL.
5. Store at 4°C. Medium is stable for only 1–2 weeks.

Accessory Products

Introduction

The following products may be used with the pTrcHis2 vectors. For details, visit www.invitrogen.com or contact **Technical Support** (page 12).

Product	Quantity	Catalog no.
ProBond™ Purification System	6 purifications	K850-01
ProBond™ Resin	50 mL	R801-01
	150 mL	R801-15
Purification Columns	50	R640-50
One Shot® Top 10 Electrocomp™ Cells	10 × 50 µL	C4040-50
One Shot® Top 10 Chemically Competent Cells	10 × 50 µL	C4040-10

Primers

For your convenience, Invitrogen offers a custom primer synthesis service. Visit www.invitrogen.com for more details.

Antibodies for Detection

Invitrogen offers the Anti-*myc* or Anti-His(C-term) antibodies to detect your recombinant fusion protein. Horseradish peroxidase (HRP)- and alkaline phosphatase (AP)-conjugated antibodies are available for convenient one-step detection.

Antibody	Epitope	Catalog no.
Anti-*myc*	Detects a 10 amino acid epitope derived from *c-myc* (Evan *et al.*, 1985): EQKLISEEDL	R950-25
Anti-*myc*-HRP		R951-25
Anti-*myc*-AP		R952-25
Anti-His(C-term)	Detects the C-terminal polyhistidine tag (requires the free carboxyl group for detection) (Lindner et al., 1997): HHHHHH-COOH	R930-25
Anti-His(C-term)-HRP		R931-25
Anti-His(C-term)-AP		R932-25

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

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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MSDS

Material Safety Data Sheets (MSDSs) are available on our website at www.invitrogen.com/msds.

Certificate of Analysis

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