Invitrogen™
life technologies

Instruction Manual

Expressway™ Plus Expression System

For high-yield, cell-free protein synthesis

Catalog no. K9900-10, K9900-20, K9900-30

Version A
April 21, 2003
25-657

Table of Contents

Procedure for Experienced Users

Introduction

This quick reference sheet is provided for experienced users of the Expressway™ Plus Expression System. If you are a first time user of the Expressway™ Plus Expression System, we recommend following the detailed protocols in the manual.

Step	Action
Generate the DNA Template	For optimal expression in the Expressway™ Plus System, clone your gene of interest into a pEXP-DEST or other T7 expression vector or use PCR to generate a DNA template containing the elements discussed on page 3.
Purify the DNA Template	Use your method of choice or a commercially available kit to purify your plasmid or linear DNA template. Resuspend purified DNA in 1X TE Buffer or water such that the final concentration is greater than 500 ng/µl. Take care to avoid contamination of your DNA template with ethanol, salt, or RNases.
Perform the Protein Synthesis Reaction	1. For each sample, add the following reagents to a 2.0 ml screw-cap tube on ice. Remember to include one of the positive controls supplied with the kit in your experiment. IVPS Plus *E. coli* Extract 20 µl 2.5X IVPS Plus *E. coli* Reaction Buffer 20 µl T7 Enzyme Mix 1 µl 75 mM Methionine 1 µl Total Volume 42 µl **Note:** To generate radiolabeled proteins, add 2 µl ^{35}S Methionine to the reaction in addition to the 1 µl of unlabeled 75 mM Methionine (included to reduce background count levels). 2. Add DNA (1 µg of plasmid DNA or 2-3 µg of linear DNA) to the reaction tube. 3. Bring the final reaction volume to 50 µl with DNase/RNase-free water. 4. Gently vortex for 3 seconds to mix. 5. Place tubes in a microcentrifuge and briefly centrifuge. 6. Shake tubes at 1,400 rpm in a thermomixer at 37°C for 2 hours. **Alternative:** Incubate tubes at 37°C for 2 hours in a standard shaking incubator (275-325 rpm) or in a water bath. 7. Add 5 µl of RNase A to the 50 µl reaction and vortex briefly. 8. Place tubes in a microcentrifuge and briefly centrifuge. 9. Shake tubes at 1,400 rpm in a thermomixer at 37°C for 15 minutes. **Alternative:** Incubate tubes at 37°C for 15 minutes in a standard shaking incubator (275-325 rpm) or in a water bath. 10. Place tubes in a microcentrifuge and briefly centrifuge. Place the reactions on ice and proceed to analyze samples.
Analyze Sample	Use any method of choice including Coomassie® blue staining, Western blot, or activity assay to analyze your sample. If you plan to use Coomassie® blue staining or Western blot to analyze your sample, precipitate the proteins with acetone prior to performing polyacrylamide gel electrophoresis (see page 9).

Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Amount	Catalog no.
Expressway™ Plus Expression System	20 reactions	K9900-10
Expressway™ Plus Expression System		
with pEXP1-DEST	20 reactions	K9900-20
with pEXP2-DEST	20 reactions	K9900-30

Shipping/Storage

The Expressway™ Plus Expression System is shipped on dry ice. Upon receipt, you may store the kit at -80°C or store the components as detailed below.

Kit Contents

The following reagents are supplied in the Expressway™ Plus Expression System. Store components as detailed below.

Item	Composition	Amount	Storage
IVPS Plus *E. coli* Extract	Proprietary	4 x 100 µl	-80°C
2.5X IVPS Plus *E. coli* Reaction Buffer	Proprietary	400 µl	-80°C
75 mM Methionine	4 mM KOH	20 µl	-20°C or -80°C
DNase/RNase-Free Distilled Water	---	400 µl	-20°C or -80°C
RNase A	1 mg/ml in DNase/RNase-free water	100 µl	-20°C or -80°C
T7 Enzyme Mix	Proprietary	20 µl	-80°C -20°C after initial use
pT7/CAT Control Plasmid	Lyophilized in TE buffer, pH 8.0	10 µg	-20°C or -80°C
pEXP1-GW/*lac*Z Control Plasmid	Lyophilized in TE buffer, pH 8.0	10 µg	-20°C or -80°C
2 ml Screw-Cap Tubes	--	20	Room Temperature

Product Qualification

Each lot of the Expressway™ Plus Expression System is functionally tested for protein generation by incorporation of ^{35}S-methionine. A 50 µl Expressway™ Plus protein synthesis reaction must yield greater than 18 µg of CAT protein from the pT7/CAT control plasmid.

Accessory Products

Accessory Products

DNase/RNase-free water and other reagents suitable for use with the Expressway™ Plus Expression System are available separately from Invitrogen. Ordering information is provided below.

Item	Quantity	Catalog no.
pEXP1-DEST Gateway® Vector Kit	6 µg	V960-01
pEXP2-DEST Gateway® Vector Kit	6 µg	V960-02
DNase/RNase-Free Distilled Water	500 ml	10977-015
S.N.A.P.™ MiniPrep Kit	25 reactions	K1900-01
Coomassie Brilliant Blue R®-250 Protein Stain	10 g	15528-011
β-Gal Assay Kit	100 reactions	K4155-01
β-Gal Antiserum*	--	R901-25
CAT Antiserum*	--	R902-25

*The amount of antibody supplied is sufficient for 25 Western blots.

Detecting Control Proteins

You may detect β-galactosidase expression from the pEXP1-GW/*lac*Z control plasmid using an antibody to the appropriate epitope tag (see table below). Ordering information is provided below.

The amount of antibody supplied is sufficient for 25 Western blots.

Item	Epitope	Catalog no.
Anti-Xpress™ Antibody	Detects the 8 amino acid Xpress™ epitope: DLYDDDDK	R910-25
Anti-Xpress™-HRP Antibody		R911-25
Anti-HisG Antibody	Detects the N-terminal poly-histidine (6xHis) tag followed by glycine: HHHHHHG	R940-25
Anti-HisG-HRP Antibody		R941-25
Anti-HisG-AP Antibody		R942-25

Coomassie Brilliant Blue R® is a registered trademark of Imperial Chemical Industries PLC

Introduction

Overview

Introduction

The Expressway™ Plus Expression System ("Expressway™ Plus System") is designed for T7-based, *in vitro* transcription and translation of target DNA to protein in a single tube. The Expressway™ Plus System has been enhanced to provide higher levels of functional protein over the original Expressway™ *In Vitro* Protein Synthesis System. The Expressway™ Plus System uses extract from an *E. coli* strain to drive the reaction and a transcription/translation mix that allows for optimal protein production (up to 50 µg protein per 50 µl reaction, depending on the protein of interest) in about 2 hours. For higher yields, the reaction can be scaled up to a higher volume or the reaction can be extended up to 4 hours.

Components of the System

The major components of the Expressway™ Plus System include:

- S30 *E. coli* extract (Zubay, 1973)
- T7 Enzyme Mix containing T7 RNA polymerase (Studier *et al.*, 1990)
- Optimized reaction buffer composed of an ATP regenerating system and all the required amino acids except methionine (Kim *et al.*, 1996; Lesley *et al.*, 1991; Pratt, 1984)
- Methionine provided separately for optimization of radiolabeling assays
- pT7/CAT and pEXP1-GW/*lac*Z plasmids for use as positive controls for protein synthesis in the Expressway™ Plus System

Applications

The Expressway™ Plus System is suitable for use in the following applications:

- Characterizing proteins
- Analyzing mutants
- Verifying cloned gene products
- Producing analytical quantities of protein or radiolabeled protein
- Producing proteins which are toxic to cells

Experimental Outline

The table below describes the major steps necessary to synthesize your recombinant protein of interest using the Expressway™ Plus Expression System. Refer to the specified pages for details to perform each step.

Step	Action	Pages
1	Generate the DNA template.	2-4
2	Purify your DNA template.	5
3	Perform the protein synthesis reaction.	6-8
4	Analyze your sample using polyacrylamide gel electrophoresis, Western blot analysis, or activity assay.	9-13

Methods

Generating the DNA Template

Introduction

Successful use of the Expressway™ Plus Expression System requires only the addition of a DNA template containing the gene of interest placed within the proper context of transcription/translation regulatory elements including a bacteriophage T7 RNA polymerase promoter ("T7 promoter"), prokaryotic Shine-Dalgarno ribosome binding site (RBS), ATG initiation codon, stop codon, and T7 terminator. However, protein yield can be significantly enhanced if the DNA template is optimally configured. Guidelines are provided to produce your DNA template in an optimal configuration for protein expression in the Expressway™ Plus System.

Factors Affecting Protein Yield

The yield of protein produced in *in vitro* transcription and translation systems is generally dependent on many factors, including:

- The size of the protein
- The sequence of the gene of interest
- The spacing of the T7 promoter and the gene of interest in the DNA template
- The quality of the DNA template
- Stability of mRNA

Recommendations and guidelines to generate a DNA template with the optimal configuration and to purify the DNA template are provided in this section. The size of the protein and its sequence will vary depending on your gene of interest. Any variability in protein yield due to these two factors will require empiric experimentation to optimize expression conditions.

DNA Templates

The following DNA templates may be used in the Expressway™ Plus System.

- Supercoiled plasmid DNA (recommended for most applications for highest protein yields)
- Linear DNA
- PCR product

For proper expression in the Expressway™ Plus System, all templates **must** contain the T7 promoter, an initiation codon, and a prokaryotic Shine-Dalgarno ribosome binding site (RBS) upstream of the gene of interest. See the next page for a discussion of template optimization.

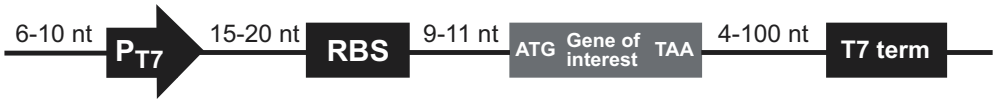
continued on next page

Generating the DNA Template, continued

Optimal Configuration of DNA Template

Optimized expression vectors are provided with the Expressway™ Plus System with pEXP1-DEST or the Expressway™ Plus System with pEXP2-DEST (Catalog nos. K9900-20 and K9900-30, respectively), however, other expression vectors may be used. If you are designing your own expression construct, we recommend generating a DNA template that contains the following elements (see the figure below for reference). See the next page for an example.

- Gene of interest placed downstream of a T7 promoter and a ribosome binding site (RBS). The gene of interest **must** contain an ATG initiation codon and a stop codon.
- Sequence upstream of the T7 promoter containing a minimum of 6-10 nucleotides (nt) for efficient promoter binding (**required** for linear PCR products). This sequence need not be specific.
- Sequence following the T7 promoter containing a **minimum** of 15-20 nt which forms a potential stem-and-loop structure as described by Studier *et al*., 1990 (see **T7 Expression Vectors**, below for more information).
- Sequence of 9-11 nt between the RBS and the ATG initiation codon for optimal translation efficiency of the protein of interest. This sequence need not be specific.
- A T7 terminator located 4-100 nt downstream of the gene of interest for efficient transcription termination and message stability. For the sequence of the T7 terminator, see the next page.



T7 Expression Vectors

Many T7-based expression vectors contain a T7 promoter, RBS, and T7 terminator with the suitable spacing and sequence configuration for optimal expression of protein in the Expressway™ Plus System. In general, T7-based expression vectors that contain the bacteriophage ϕ10-s10 segment (*i.e.* ϕ10 promoter and the translation initiation region for the gene 10 protein) are recommended for use (see Studier *et. al.*, 1990 for a list). The ϕ10-s10 segment contains a region that forms a hypothetical stem-and-loop structure as described by Studier *et al.*, 1990. Examples of T7-based expression vectors containing this configuration include:

- pEXP1-DEST and pEXP2-DEST (supplied with Catalog nos. K9900-20 and K9900-30, respectively)
- pCR®T7/CT-TOPO® (Invitrogen, Catalog no. K4210-01)
- pCR®T7/NT-TOPO® (Invitrogen, Catalog no. K4200-01)
- pRSET (Invitrogen, Catalog no. V351-20)

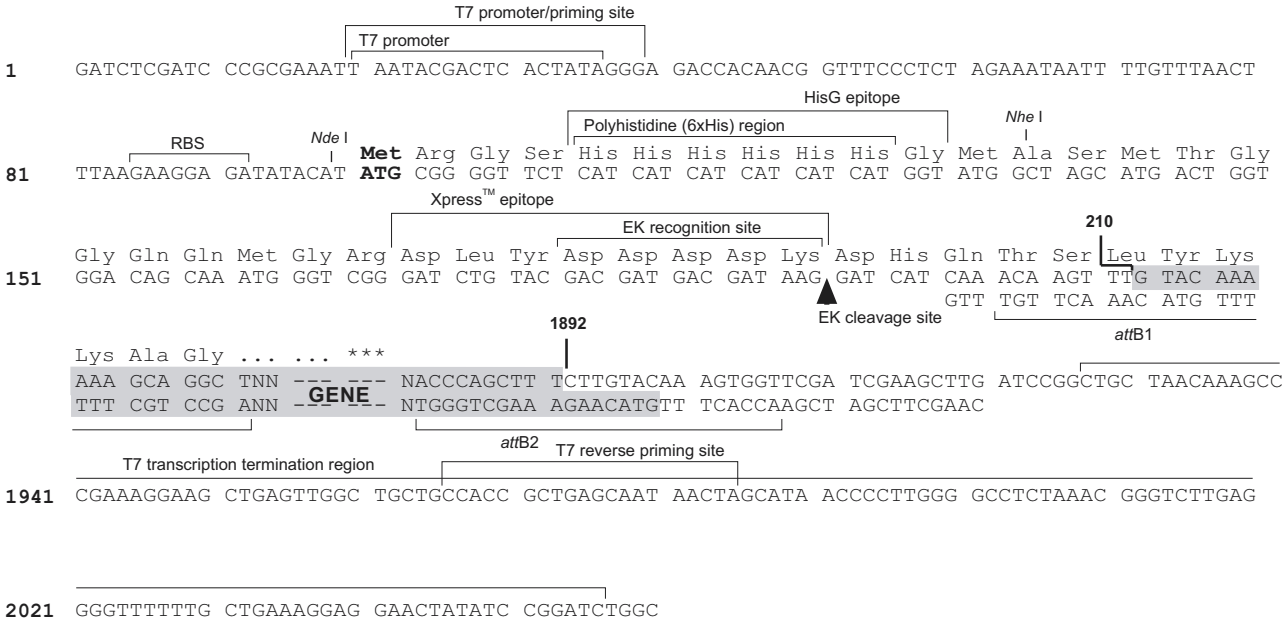
Note: T7 expression vectors that contain the T7*lac* promoter (*e.g.* pET101/D-TOPO® available from Invitrogen) are suitable for use in the Expressway™ Plus System although protein yield may be reduced. In these vectors, the *lac* operator replaces the region containing the hypothetical stem-and-loop structure.

continued on next page

Generating the DNA Template, continued

Example

The T7-based pEXP1-DEST and pEXP2-DEST vectors (supplied with Catalog nos. K9900-20 and K9900-30, respectively) allow you to generate an expression clone containing your gene of interest in an optimal configuration for expression in the Expressway™ Plus System. To illustrate the points discussed on the previous page, the region surrounding the gene of interest after generating an expression clone with pEXP1-DEST is diagrammed below.



Generating an Expression Clone

If you are using the Expressway™ Plus System with pEXP1-DEST or the Expressway™ Plus System with pEXP2-DEST, you may generate an expression clone by recombination of an entry clone containing your gene of interest with the appropriate destination vector using the Gateway® Technology. For more information about the pEXP1-DEST and pEXP2-DEST vectors, refer to the pEXP1-DEST and pEXP2-DEST Gateway® Vector Kits manual supplied with the vectors. For more information about the Gateway® Technology, creating an entry clone, and performing the LR recombination reaction, refer to the Gateway® Technology manual which is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (page 20).

continued on next page

Generating the DNA Template, continued

Purifying the DNA Template

Once you have generated the DNA template, you must purify the DNA before proceeding to the protein synthesis reaction. You may use a variety of methods to purify your DNA template including commercial DNA purification kits (see below) or CsCl gradient centrifugation. For protocols to purify DNA, refer to published reference sources (Ausubel *et al.*, 1994; Sambrook *et al.*, 1989). When purifying your DNA template, keep the following in mind:

- **Do not** gel-purify your DNA template. Purified DNA solution obtained from agarose gels significantly inhibits the protein synthesis reaction. For PCR products or linear DNA, we recommend using a suitable purification kit.
- Ammonium acetate is not recommended for use in DNA precipitation as any residual contamination may inhibit translation. Use sodium acetate.
- Purified DNA **must** be free of RNases (wear gloves and use RNase-free reagents when preparing DNA).
- Purified DNA should be free of excess ethanol or salt as both can inhibit translation.

  Note: Ethanol precipitated DNA should be carefully washed with 70% ethanol to remove excess salt and dried.
- Purified DNA should be resuspended in 1X TE Buffer or water such that the final concentration is at a minimum of 500 ng/µl.

Important

Reminder: Do not gel-purify your DNA template. Purified DNA solution obtained from agarose gels significantly inhibits the protein synthesis reaction. For PCR products or linear DNA, we recommend using a suitable purification kit.

For rapid, resin-based isolation of purified plasmid DNA, we recommend using the S.N.A.P.™ MiniPrep Kit (Catalog no. K1900-01) available from Invitrogen. Other commercial DNA purification kits are suitable.

Performing the Protein Synthesis Reaction

Introduction

Once you have obtained purified template DNA, you are ready to synthesize your recombinant protein using the Expressway™ Plus System. General guidelines and instructions to produce your recombinant protein are provided in this section.

Important

RNase contamination may affect protein yield. To reduce the chances of RNase contamination, wear gloves and use RNase-free reagents (*i.e.* microcentrifuge tubes and pipette tips) when performing the protein synthesis reaction.

2 ml Tubes

We recommend performing the protein synthesis reactions in the 2 ml screw-cap tubes provided with the kit. Sterile 0.5 or 1.5 ml microcentrifuge tubes are also suitable but may result in lower yields of protein.

Incubation Conditions

To obtain the optimal yield of protein, we recommend using an Eppendorf Thermomixer (Fisher, Catalog no. 05-400-200) to shake your sample(s) at 37°C during the protein synthesis reaction (see Steps 6-9 in the protocol on page 8).

If a thermomixer is unavailable, you may use one of the following:

- Standard shaking incubator
- Standard shaking water bath
- Non-shaking water bath

We do not recommend using a non-shaking incubator because it produces a less stable and less consistent temperature environment. If you use the 2 ml tubes provided with the kit and one of the thermomixer alternatives listed above, the protein yield will be comparable to that obtained using the thermomixer.

Positive Control

The pT7/CAT and pEXP1-GW/*lac*Z vectors are provided in the kit for use as positive controls for protein expression. pT7/CAT allows expression of the chloramphenicol acetyltransferase (CAT) protein. pEXP1-GW/*lac*Z allows expression of an N-terminally tagged galactosidase fusion protein. Both proteins can be detected by Western blot or functional assay. For details about each vector, refer to pages 18 and 19. To propagate and maintain each plasmid:

1. Resuspend the vector in 20 µl of sterile water to prepare a 0.5 µg/µl stock solution.
2. Use the stock solution to transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α™-T1^R, or equivalent. Use 10 ng of plasmid for transformation.
3. Select transformants on LB agar plates containing 50-100 µg/ml ampicillin.
4. Prepare a glycerol stock of a transformant containing plasmid for long-term storage.

continued on next page

Performing the Protein Synthesis Reaction, continued

Materials to Have on Hand

You should have the following materials on hand before beginning:

- DNA Template (purified; resuspended in TE or water at a concentration greater than 500 ng/µl)
- pT7/CAT or pEXP1-GW/*lac*Z control plasmid, optional (supplied with the kit; resuspended to 0.5 µg/µl in sterile water)
- IVPS Plus *E. coli* Extract (supplied with the kit; thaw on ice)
- 2.5X IVPS Plus *E. coli* Reaction Buffer (supplied with the kit; thaw on ice)
- T7 Enzyme Mix (supplied with the kit; keep on ice; store at -20°C after initial use)
- 75 mM Methionine (supplied with the kit)
- ^{35}S Methionine, if needed (3,000 Ci/mmol; 15 µCi/µl)
- DNase/RNase-free water (supplied with the kit)
- RNase A (supplied with the kit; do not thaw until needed)
- 2 ml screw-cap tubes, one for each sample (supplied with the kit)
- Thermomixer (recommended), standard shaking incubator (set to 37°C), or water bath (set to 37°C)

Note

Upon thawing the 2.5X IVPS Plus *E. coli* Reaction Buffer, you may notice some precipitate in the bottom of the tube. Gently flick the tube several times with your finger to mix and allow the precipitate to go back into solution. **Do not** pipette the buffer up and down or place the tube in a warm (greater than 37°C) water bath as this may result in loss of activity of the Reaction Buffer. Note that the solution may remain cloudy but is suitable for use.

Important

Four tubes containing 100 µl each of IVPS Plus *E. coli* Extract and one tube containing 400 µl of 2.5X IVPS Plus *E. coli* Reaction Buffer are provided with the kit. Depending on the number of protein synthesis reactions performed, you may not use the entire contents of a tube in a single experiment. In this case, we recommend you do the following:

1. Thaw on ice the 2.5X IVPS Plus *E. coli* Reaction Buffer and the appropriate number of tubes of IVPS Plus *E. coli* Extract.
2. Mix the 2.5X IVPS Plus *E. coli* Reaction Buffer (see **Note** above).
3. Remove the amount of IVPS Plus *E. coli* Extract and IVPS Plus *E. coli* Reaction Buffer needed for Step 1 of the protein synthesis reaction (see next page) and return tubes to a -80°C freezer.

   Note: To prevent contamination, use RNase-free, sterile pipette tips and wear gloves when removing the *E. coli* Extract and Reaction Buffer from the tubes.

Do not store the IVPS Plus *E. coli* Extract or 2.5X IVPS Plus *E. coli* Reaction Buffer at -20°C or room temperature as this may result in loss of activity. Both the *E. coli* Extract and Reaction Buffer may undergo multiple rounds of freeze/thaw cycles without loss of activity.

continued on next page

Performing the Protein Synthesis Reaction, continued

Performing the Protein Synthesis Reaction

Use the protocol below to synthesize your protein from the DNA template.

1. For each sample, add the following reagents to a 2.0 ml screw-cap tube on ice. For multiple samples, scale up the volume of each reagent accordingly and aliquot the cocktail into individual 2.0 ml screw-cap tubes.

IVPS Plus *E. coli* Extract	20 µl
2.5X IVPS Plus *E. coli* Reaction Buffer	20 µl
T7 Enzyme Mix	1 µl
75 mM Methionine	1 µl
Total Volume	42 µl

   Note: To generate radiolabeled proteins, add 2 µl ^{35}S Methionine to the reaction in addition to the 1 µl of unlabeled 75 mM Methionine (included to reduce background count levels). If you wish to increase the specificity of ^{35}S Methionine incorporation, reduce or eliminate the amount of unlabeled methionine added to the reaction. Include a negative control (no DNA) to determine background.

2. Add the following amount of DNA to each 2 ml tube.
   - For plasmid DNA templates, add 1 µg
   - For linear DNA templates or PCR products, add 2-3 µg
   - For control DNA, add 1 µg
3. Bring the final reaction volume to 50 µl with DNase/RNase-free water.
4. Gently vortex for 3 seconds to mix.
5. Place tubes in a microcentrifuge and briefly centrifuge.
6. Shake tubes at 1,400 rpm in a thermomixer at 37°C for 2 hours. **Alternative:** Incubate tubes at 37°C for 2 hours in a standard shaking incubator (275-325 rpm) or in a water bath.

   Note: It is possible to incubate tubes for up to 4 hours to obtain greater protein yield. You may also incubate tubes at temperatures as low as 25°C to decrease the rate of protein synthesis and to promote proper folding. If you will be incubating tubes at temperatures lower than 37°C, we recommend extending the incubation time to 4 hours.
7. Add 5 µl of RNase A to the 50 µl reaction and vortex briefly.
8. Place tubes in a microcentrifuge and briefly centrifuge.
9. Shake tubes at 1,400 rpm in a thermomixer at 37°C for 15 minutes. **Alternative:** Incubate tubes at 37°C for 15 minutes in a standard shaking incubator (275-325 rpm) or in a water bath.
10. Place tubes in a microcentrifuge and briefly centrifuge. Place the reaction on ice and proceed to **Analyzing Samples**, next page.

Analyzing Samples

Introduction

Once you have performed the protein synthesis reaction, you may use any method of choice to analyze your sample. Generally, sufficient protein is produced for analysis on a Coomassie®-stained protein gel, by Western blot analysis, by enzymatic activity, or by affinity purification (if affinity tag is present), however, expression levels may vary depending on the nature of your protein and the configuration of the DNA template (see page 3 for more details). If you plan to analyze your sample using polyacrylamide gel electrophoresis, note that you should first precipitate the proteins with acetone to remove background smearing. A protocol for acetone precipitation and other general guidelines for gel electrophoresis are provided in this section.

If you have included radiolabeled methionine in the protein synthesis reaction, you may use TCA precipitation to determine the amount of radiolabeled methionine incorporated and to calculate the yield of protein (see pages 11-13).

Materials Needed

You should have the following materials on hand before proceeding:

- Acetone (room temperature)
- SpeedVac® concentrator (Thermo Savant)
- 1X SDS-PAGE sample buffer (see page 17 for a recipe)
- Appropriate polyacrylamide gel to resolve your protein of interest (see the next page)
- Coomassie® blue stain

Acetone Precipitation

Before starting, prepare an SDS-PAGE gel or use one of the pre-cast polyacrylamide gels available from Invitrogen (see the next page) to analyze your samples. Use the following protocol to precipitate your proteins prior to loading on the polyacrylamide gel.

1. Add 5 µl of the protein reaction product from Step 10, previous page, to 20 µl of acetone. Mix well.
2. Centrifuge for 5 minutes at room temperature in a microcentrifuge at 12,000 rpm.
3. Carefully remove the supernatant, taking care not to disturb the protein pellet.
4. Dry the sample in a SpeedVac® for 15 minutes. **Note:** Alternatively, you may air dry the sample for 1 hour at room temperature.
5. Resuspend pellet in 20 µl of 1X SDS-PAGE sample buffer.
6. Heat at 70-80°C for 10-15 minutes and centrifuge briefly. Proceed to **Polyacrylamide Gel Electrophoresis**, next page. **Note:** Alternatively, samples may be stored at -20°C until needed.

continued on next page

®SpeedVac is a registered trademark of Thermo Savant

Analyzing Samples, continued

Polyacrylamide Gels Available from Invitrogen™

To facilitate separation and visualization of your recombinant protein by polyacrylamide gel electrophoresis, a wide range of pre-cast NuPAGE® and Novex® Tris-Glycine polyacrylamide gels and electrophoresis apparatus are available from Invitrogen. In addition, Invitrogen carries a large selection of molecular weight protein standards and staining kits. For more information about the appropriate gels, standards, and stains to use to visualize your recombinant protein, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 20).

Polyacrylamide Gel Electrophoresis

1. Load 5-10 µl of the sample from Step 6, previous page on an SDS-PAGE gel and electrophorese at 120V. You may save your sample by storing at -20°C, if desired.
2. Depending on your assay of choice, perform the following. Refer to page 12 for sample gels and autoradiographs.

If you are…	Then…
Visualizing your protein using Coomassie® blue staining	Stain gel with Coomassie® blue stain. **Note:** For radiolabeled proteins, the signal may be enhanced by placing the gel in a commercially available reagent that enhances the signal. Dry the gel and expose to x-ray film for 1-4 hours.
Analyzing your protein by Western blot	Transfer proteins electrophoretically to a suitable membrane and use an appropriate antibody to detect the protein of choice.

Assay for CAT

If you use pT7/CAT as a positive control for protein expression, you may assay for CAT protein using CAT Antiserum available separately from Invitrogen (see page viii for ordering information). Other commercial kits are available for assaying CAT expression. The molecular weight of the CAT protein is approximately 25 kDa.

Assay for β-galactosidase

If you use pEXP1-GW/*lac*Z as a positive control for protein expression, you may assay for β-galactosidase protein using β-Gal Antiserum or the β-Gal Assay Kit available separately from Invitrogen (see page viii for ordering information). Because the β-galactosidase protein is fused to the N-terminal 6xHis and Xpress™ tag, you may also detect the fusion protein using antibodies against the appropriate epitope (see page viii for ordering information). The molecular weight of the β-galactosidase fusion protein is approximately 122 kDa.

continued on next page

Analyzing Samples, continued

Determining Total Counts Incorporated

For radiolabeled reactions, we recommend using TCA precipitation to determine the amount of ^{35}S methionine incorporated. A protocol is provided below.

Determining Total Counts

1. Mix and spot 5 µl of each radiolabeled reaction from Step 10, page 8 on a glass microfiber filter (Type GF/C; Whatman, Catalog no. 1822-021).
2. Set aside and let dry. **Do not** wash or TCA precipitate these filters.

Performing TCA Precipitation

1. Mix and spot 5 µl of each radiolabeled reaction from Step 10, page 8 on a separate set of individual glass fiber (GF/C) filters and allow to air dry for approximately 5-10 seconds.
2. Place filter in a beaker and wash once with cold 10% TCA/1% sodium pyrophosphate for 10 minutes at room temperature while shaking gently (use approximately 10-20 ml per filter).
3. Wash with 5% TCA for 5 minutes at room temperature while shaking gently. Repeat wash.
4. Rinse filters with methanol to facilitate drying.
5. Allow filters to dry, place in scintillation vials, and add scintillation fluid.
6. Count samples in a scintillation counter.
7. Proceed to **Calculating the Yield of Protein**, below, to determine your protein yield.

Calculating the Yield of Protein

If you generated radiolabeled proteins using 2 µl of ^{35}S methionine (3,000 Ci/mmol, 15 µCi/µl) **and** included 1 µl of unlabeled 75 mM Methionine in the protein synthesis reaction (see page 8), you may calculate the yield of protein as shown below. Otherwise, you will need to calculate the pmoles of methionine present in your specific reaction. Refer to page 13 for a sample calculation.

Before beginning, you will need to determine the total counts incorporated using TCA precipitation (see above).

Total pmoles of methionine (includes radiolabeled and non-radiolabeled methionine) = 1,500 pmoles/µl (or 75,000 total pmoles for a 50 µl reaction)

Total counts:
$$\text{total cpm per 5 µl spotted} \times \frac{\text{total reaction volume}}{5}$$

Specific activity:
$$\frac{\text{total counts}}{\text{pmoles of methionine}}$$

pmoles methionine incorporated:
$$\frac{\left[(\text{TCA precipitable counts - background}) \times \frac{50}{5}\right]}{\text{specific activity}}$$

pmoles of protein:
$$\frac{\text{pmoles of methionine incorporated into protein}}{\text{number of methionines in protein}}$$

Yield of protein:
$$\text{pmoles of protein} \times \text{molecular weight of protein}$$

Sample Protein Synthesis Reactions

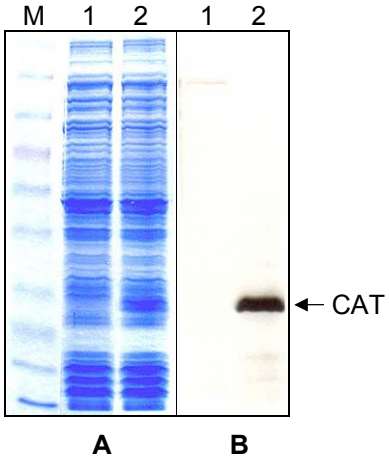
Introduction

Sample Coomassie®-stained gels and autoradiographs of Expressway™ Plus protein synthesis reactions using the pT7/CAT and pEXP1-GW/*lac*Z control plasmids are provided below. A synthesis reaction using pEXP2-GW/*lac*Z, a control plasmid provided with Catalog no. K9900-30, is also included in Figure 2. Refer to the next page for a sample calculation of the protein yield obtained from the pT7/CAT reaction.

Sample Results

Standard 50 µl Expressway™ Plus reactions were performed using the protocol on page 8 with ^{35}S methionine and the following control plasmids: pT7/CAT (Figure 1), pEXP1-GW/*lac*Z (Figure 2), and pEXP2-GW/*lac*Z (Figure 2).

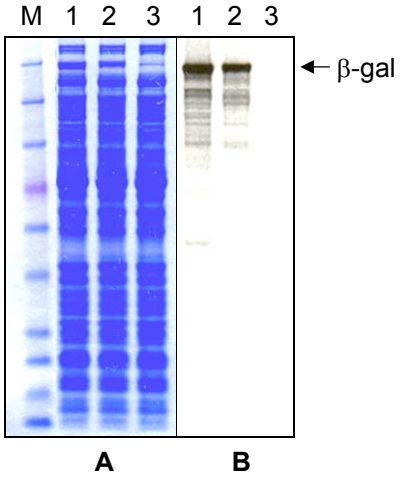
Figure 1: Radiolabeled Protein Synthesis from pT7/CAT



1.25 µl of each protein synthesis reaction was run on a Novex® 4-12% Tris-Glycine gel and stained with Coomassie® blue stain (Panel A) and exposed to x-ray film (Panel B).

Lane M: BenchMark™ Pre-Stained Protein Ladder
Lane 1: No DNA
Lane 2: pT7/CAT

Figure 2: Radiolabeled Protein Synthesis from pEXP1-GW/*lac*Z and pEXP2-GW/*lac*Z



1.25 µl of each protein synthesis reaction was run on a NuPAGE® 4-12% Bis-Tris gel and stained with Coomassie® blue stain (Panel A) and exposed to x-ray film (Panel B).

Lane M: BenchMark™ Pre-Stained Protein Ladder
Lane 1: pEXP1-GW/*lac*Z
Lane 2: pEXP2-GW/*lac*Z
Lane 3: No DNA

continued on next page

Sample Protein Synthesis Reactions, continued

Sample Calculation for Protein Yield

The sample calculation below illustrates how to use the data obtained from the TCA precipitation procedure, page 11, to calculate the yield of protein.

Example: We wish to calculate the yield of chloramphenicol acetyltransferase (CAT) protein obtained from the pT7/CAT reaction shown in Figure 1, previous page. For the 50 µl reaction, we added 2 µl of ^{35}S methionine (3,000 Ci/mmol, 15 µCi/µl) and 1 µl of the unlabeled 75 mM Methionine provided with the kit.

1. We know the following:
   - The CAT protein has a molecular weight of 25 kDa (0.025 µg/pmole) and contains 9 methionines.

     Note: If your protein is fused to an N- or C-terminal tag, you will need to account for any additional methionines that are present in the tags.
   - There are 75,000 total pmoles of methionine in a 50 µl reaction.
2. Using the TCA precipitation protocol, we obtained the following values after scintillation counting:

Sample	Radioactive Counts (cpm)
Total counts (5 µl spotted):	8.7×10^5 cpm
Background TCA precipitable counts (No DNA; 5 µl spotted):	3.4×10^3 cpm
CAT TCA precipitable counts (5 µl spotted):	9.8×10^4 cpm

3. Determine total counts in the reaction:

$$8.7 \times 10^5 \text{ cpm in } 5\,\mu\text{l} \times \frac{50}{5} = 8.7 \times 10^6 \text{ cpm in } 50\,\mu\text{l reaction}$$

4. Calculate the specific activity:

$$\frac{8.7 \times 10^7 \text{ cpm}}{75000 \text{ pmoles}} = 116 \text{ cpm/pmoles methionine}$$

5. Calculate the pmoles methionine incorporated:

$$\frac{\left[\left(9.8 \times 10^4 \text{ cpm} - 3.4 \times 10^3 \text{ cpm}\right) \times \frac{50}{5}\right]}{116 \text{ cpm/pmoles methionine}} = 8155 \text{ pmoles methionine}$$

6. Calculate the pmoles of protein obtained:

$$\frac{8155 \text{ pmoles methionine}}{9 \text{ methionines/protein}} = 906 \text{ pmoles protein}$$

7. Calculate the yield of protein obtained:

$$906 \text{ pmoles protein} \times 0.025\ \mu\text{g/pmole} = 22.7\ \mu\text{g protein}$$

Troubleshooting

Introduction

The table below and on the next page lists some potential problems and possible solutions that you may use to help you troubleshoot your *in vitro* transcription and translation experiment.

Problem	Reason	Solution
Low or no yield of target protein (but control reaction produces protein)	DNA template not optimally configured	• Use one of the pEXP-DEST vectors or follow the guidelines on pages 2-3 to clone your gene of interest into a T7 expression vector with the optimal configuration. • Make sure that the ATG initiation codon is in the proper context for expression (*i.e.* check spacing and placement after the RBS). • For linear DNA templates, make sure that at least 6-10 additional nucleotides are present upstream of the T7 promoter.
	DNA template not pure • Contaminated with ethanol, sodium salt, or ammonium acetate • Contaminated with RNases	• Prepare new DNA template taking care to remove excess ethanol and/or salt after precipitation. • Do not use ammonium acetate to precipitate DNA. Use sodium acetate. • Wear gloves and use RNase-free reagents when preparing DNA.
	DNA template purified from agarose gel	We recommend using a commercial DNA purification kit to purify your DNA template. See page 5 for additional guidelines.
	DNA concentration not optimal (generally more of a problem for linear DNA or PCR products)	• Determine the concentration of your DNA template and adjust the concentration to at least 500 ng/µl. • Titrate the amount of DNA template used in the protein synthesis reaction to determine the optimal yield.
	Protein synthesis reaction performed in a 0.5 or 1.5 ml microcentrifuge tube	For optimal protein yield, perform reactions in the 2 ml screw-cap tubes provided with the kit.
	Sample incubated in a non-shaking incubator during protein synthesis reaction	• Use a thermomixer and shake sample at 1,400 rpm (see protocol on page 8). • Incubate samples in a standard shaking incubator (275-325 rpm) or in a water bath (see recommended alternatives on page 6).
	Expression time too short	Extend expression time up to 4 hours.

continued on next page

Troubleshooting, continued

Problem	Reason	Solution
	Size of protein	• Protein yield may decrease as the size of the protein increases; optimize expression conditions. • Reduce incubation temperature to as low as 25°C for Step 6 of protocol on page 8. Extend incubation time for up to 4 hours.
	Sample not mixed before spotting on filter for TCA precipitation (radiolabeled samples only)	Mix sample before spotting on filter for TCA precipitation.
Control reaction produces no protein	Reagents have lost activity	• Store reagents at -80°C. • Store the T7 Enzyme Mix at -20°C after initial use. • Use care when freeze thawing the IVPS Plus *E. coli* Extract and 2.5X IVPS Plus *E. coli* Reaction Buffer. Follow guidelines on page 7.
	IVPS Plus *E. coli* Extract or 2.5X IVPS Plus *E. coli* Reaction Buffer contaminated	Wear gloves and use RNase-free reagents when working with the IVPS Plus *E. coli* Extract and 2.5X IVPS Plus *E. coli* Reaction Buffer to prevent contamination.
Protein has low biological activity	Improper protein folding	Reduce incubation temperature to as low as 25°C for Step 6 of protocol on page 8. Extend incubation time for up to 4 hours.
Multiple bands on the polyacrylamide gel	Proteins denatured for too long	Add 1X SDS-PAGE sample buffer to sample and heat at 70-80°C for 10-15 minutes before loading on gel.
	Old ^{35}S methionine	Use fresh ^{35}S methionine.
	Not enough SDS in the 1X SDS-PAGE sample buffer	Prepare new 1X SDS-PAGE sample buffer according to standard instructions.
	Internal ATG codons in the context of RBS-like sequences	• Check the sequence of your gene and search for potential RBSs with the proper spacing from internal methionines. • Replace the methionine **or** change RBS sequence(s) using point mutation(s).

continued on next page

Troubleshooting, continued

Problem	Reason	Solution
Smearing on the gel	Samples not precipitated with acetone	Precipitate the proteins with acetone to remove background smearing. Follow the protocol provided on page 9.
	Too much protein loaded	Reduce the amount of protein loaded on the gel.
	Gel not clean	• Rinse the gel briefly before exposing to film. • If you have stained the gel with Coomassie® blue, destain the gel in water or 50% methanol, 7.5% glacial acetic acid for 15-30 minutes before drying. If you have already destained the gel, repeat destaining procedure.
	Ethanol present in the protein synthesis reaction	Make sure that any residual ethanol is removed during DNA purification.
	Old pre-cast gels	Do not use pre-cast gels after the expiration date.

Appendix

Recipes

1X SDS-PAGE Sample Buffer

1. Combine the following reagents:

0.5 M Tris-HCl, pH 6.8	2.5 ml
Glycerol (100%)	2 ml
β-mercaptoethanol	0.4 ml
Bromophenol Blue	0.02 g
SDS	0.4 g

2. Bring the volume to 20 ml with sterile water.
3. Aliquot and freeze at -20°C until needed.

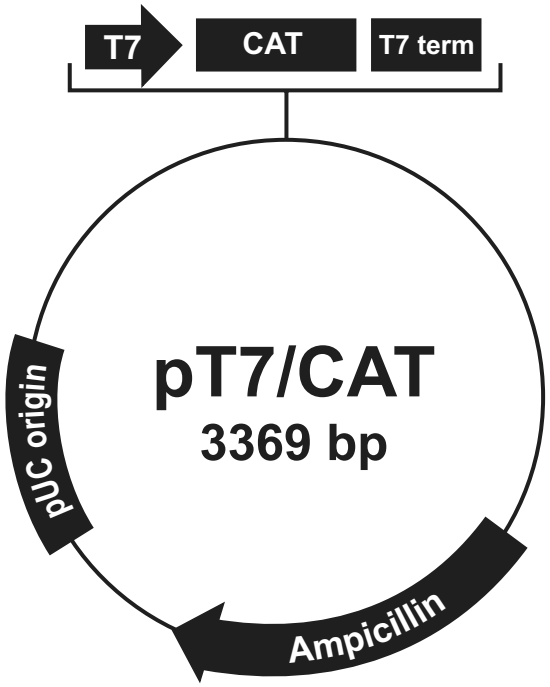
Map of pT7/CAT

Description

pT7/CAT is a 3369 bp control vector expressing the chloramphenicol acetyltransferase (CAT) protein. The CAT gene is cloned in optimal configuration for expression using the Expressway™ Plus System. The molecular weight of the CAT protein is approximately 25 kDa.

Map of pT7/CAT

The map below shows the elements of pT7/CAT. The complete sequence of the vector is available from our Web site (www.invitrogen.com) or by contacting Technical Service (page 20).



Comments for pT7/CAT
3369 nucleotides

T7 promoter: bases 31-50
Ribosome binding site: bases 96-101
CAT gene: bases 111-770
T7 transcription termination region: bases 811-939
bla promoter: bases 1050-1148
Ampicillin (*bla*) resistance gene: bases 1149-2009
pUC origin: bases 2154-2827

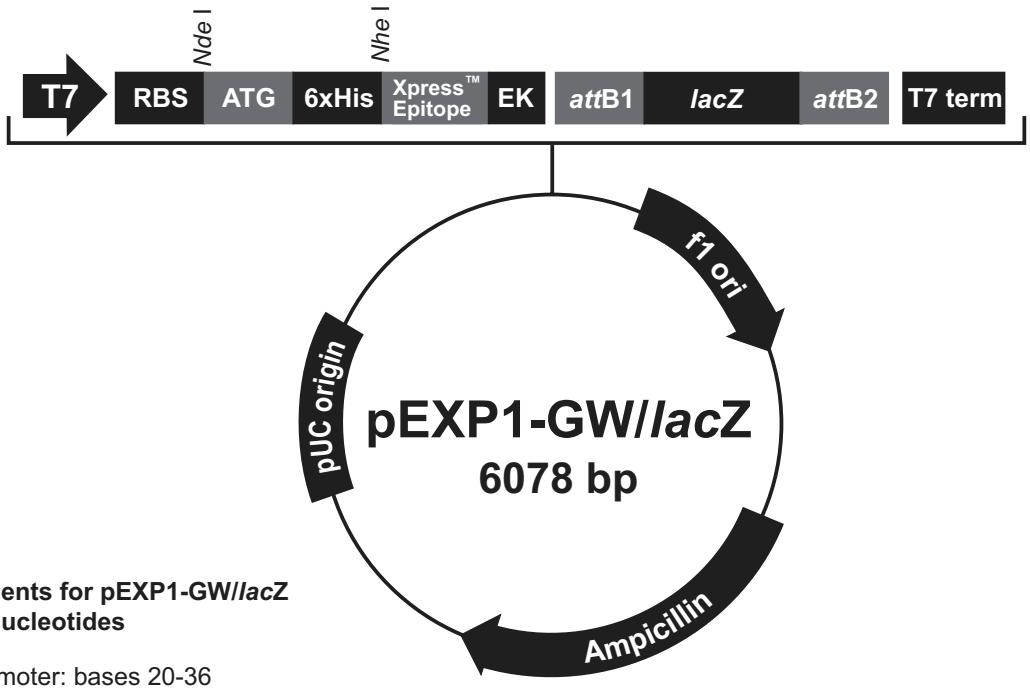
Map of pEXP1-GW/*lacZ*

Description

pEXP1-GW/*lacZ* is a 6078 bp control vector expressing β-galactosidase and was generated using the Gateway™ LR recombination reaction between an entry clone containing the *lacZ* gene and pEXP1-DEST. The *lacZ* gene is cloned in optimal configuration for expression using the Expressway™ Plus System. β-galactosidase is expressed as an N-terminal fusion protein with a molecular weight of approximately 122 kDa.

Map of pEXP1-GW/*lacZ*

The map below shows the elements of pEXP1-GW/*lacZ*. The complete sequence of the vector is available from our Web site (www.invitrogen.com) or by contacting Technical Service (page 20).



Comments for pEXP1-GW/*lacZ*
6078 nucleotides

T7 promoter: bases 20-36
T7 promoter priming site: bases 20-39
Ribosome binding site: bases 85-92
Initiation ATG: bases 100-102
Polyhistidine (6xHis) region: bases 112-129
Xpress™ epitope: bases 169-192
Enterokinase (EK) recognition site: bases 178-192
*att*B1 site: bases 202-226
lacZ gene: bases 247-3321
*att*B2 site: bases 3338-3362
T7 reverse priming site: bases 3422-3441
T7 transcription termination region: bases 3383-3512
f1 origin: bases 3583-4038
bla promoter: bases 4125-4223
Ampicillin resistance gene: bases 4224-5084
pUC origin: 5229-5902

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact Us

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

Corporate Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail:
tech_service@invitrogen.com

Japanese Headquarters:
Invitrogen Japan K.K.
Nihonbashi Hama-Cho Park Bldg. 4F
2-35-4, Hama-Cho, Nihonbashi
Tel: 81 3 3663 7972
Fax: 81 3 3663 8242
E-mail: jpinfo@invitrogen.com

European Headquarters:
Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

MSDS Requests

To request an MSDS, visit our Web site at www.invitrogen.com. On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

continued on next page

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, please contact our Technical Service Representatives.

Invitrogen warrants that all of its products will perform according to the specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order.

Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Service Representatives.

Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

Purchaser Notification

Introduction

Use of the Expressway™ Plus Expression System is covered under the licenses detailed below.

Limited Use Label License No. 19: Gateway® Cloning Products

The Gateway® Cloning Technology products and their use are the subject of one or more of U.S. Patent Nos. 5,888,732, 6,143,557, 6,171,861, 6,270,969, and 6,277,608 and/or other pending U.S. and foreign patent applications owned by Invitrogen Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). No license is conveyed under the foregoing patents to use this product with any recombination sites other than those purchased from Invitrogen Corporation or its authorized distributor. The buyer cannot modify the recombination sequence(s) contained in this product for any purpose. The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500.

continued on next page

Purchaser Notification, continued

Limited Use Label License No. 22: Vectors & Clones Encoding Histidine Hexamer

This product is licensed under U.S. Patent Nos. 5,284,933 and 5,310,663 and foreign equivalents from Hoffmann-LaRoche, Inc., Nutley, NJ and/or Hoffmann-LaRoche Ltd., Basel, Switzerland and is provided only for use in research. Information about licenses for commercial use is available from QIAGEN GmbH, Max-Volmer-Str. 4, D-40724 Hilden, Germany.

Limited Use Label License No. 133: Expressway™ Protein Expression System

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of patents owned by Invitrogen Corporation and claiming this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500.

References

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).

Kim, D. M., Kigawa, T., Choi, C. Y., and Yokoyama, S. (1996). A Highly Efficient Cell-free Protein Synthesis System from *E. coli*. Eur. J. Biochem. *239*, 881-886.

Lesley, S. A., Brow, M. A., and Burgess, R. R. (1991). Use of *in vitro* Protein Synthesis from Polymerase Chain Reaction-generated Templates to Study Interaction of *Escherichia coli* Transcription Factors with Core RNA Polymerase and for Epitope Mapping of Monoclonal Antibodies. J. Biol. Chem. *266*, 2632-2638.

Pratt, J. M. (1984). Transcription and Translation (Oxford: S.J. IRL Press).

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Second Edition (Plainview, New York: Cold Spring Harbor Laboratory Press).

Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990). Use of T7 RNA Polymerase to Direct Expression of Cloned Genes. Meth. Enzymol. *185*, 60-89.

Zubay, G. (1973). *In vitro* Synthesis of Protein in Microbial Systems. Annu. Rev. Genet. *7*, 267-287.

Notes

Notes

United States Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, California 92008
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 603 7229
Email: tech_service@invitrogen.com

European Headquarters:
Invitrogen Ltd
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Tel (Free Phone Orders): 0800 269 210
Tel (General Enquiries): 0800 5345 5345
Fax: +44 (0) 141 814 6287
Email: eurotech@invitrogen.com

International Offices:
Argentina 5411 4556 0844
Australia 1 800 331 627
Austria 0800 20 1087
Belgium 0800 14894
Brazil 0800 11 0575
Canada 800 263 6236
China 10 6849 2578
Denmark 80 30 17 40

France 0800 23 20 79
Germany 0800 083 0902
Hong Kong 2407 8450
India 11 577 3282
Italy 02 98 22 201
Japan 03 3663 7974
The Netherlands 0800 099 3310
New Zealand 0800 600 200
Norway 00800 5456 5456

Spain & Portugal 900 181 461
Sweden 020 26 34 52
Switzerland 0800 848 800
Taiwan 2 2651 6156
UK 0800 838 380
For other countries see our website

www.invitrogen.com