

PRODUCT INFORMATION

PRODUCT USAGE

Cloning of a gene to pETIA:

Below is the multiple cloning site of pETIA. To express a recombinant protein correctly, it is necessary to clone the gene of interest in frame with an N-terminal peptide of pETIA. The start codon of pETIA is boxed ATG in the below figure. Digest pETIA completely with appropriate restriction enzyme(s) to form DNA ends which can be ligated to the gene of interest. If only one restriction enzyme is used, dephosphorylation of a vector is often performed. Ligation of processed pETIA and the gene of interest can be performed by the standard procedure. The following transformation procedure should be done with non-expression hosts such as DH5 α or JM109. Recombinant plasmids derived from pETIA are selected by colony-PCR, enzyme digestion of prepared plasmids, or other methods. Sequencing of cloning portion and an insert region on the obtained plasmid is recommended to determine the correct recombinant plasmids for expression experiments.

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                T7 promoter                                XbaI
GATCCCGCGA AATTAATACG ACTCACTATA GGGAGACCAC AACGGTTTCC CTCTAGAAAT 259
AspProAlaL ysLeuIleAr gLeuThrIle GlyArgProG lnArgPhePr oSerArgAsn
                NdeI                                6xHis
AATTTTGTTF AACTTTAAGA AGGAGATATA CATATGCGGG GTTCTCATCA TCATCATCAT 319
AsnPheVal* **Leu***Gl uGlyAspIle HisMetArgG lySerHisHi sHisHisHis
                EK                                BamHI
CATGGTATGG CTAGCATGAC TGGTGGACAG CAAATGGGTC GGGACGATGA CGATAAGGAT 379
HisGlyMetA laSerMetTh rGlyGlyGln GlnMetGlyA rgAspAspAs pAspLysAsp
                KpnI                                EcoRI                                SalI                                NotI                                ↑
CCCCGGGTAC CGAGCTCGAA TTCGATTTTCG TCGACAAGCT TAGCGGCCGC CGTTTAAATCC 439
                SmaI                                SacI                                HindIII
ProArgValP roSerSerAs nSerIleSer SerThrSerL euAlaAlaAl aVal***Ser
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EK: Enterokinase recognition sequence (AspAspAspAspLys↓)

ATG: start codon

TAA: stop codon

Protein Expression Procedure :

The following protocol is a general guide for the protein expression by using T7 expression vectors, pETUA, pETBA, pETIA, pETUK, pETBK, and pETIK, coupling with an expression host *E. coli* cell, BL21(DE3) cells or BL21(DE3)pLysS cells.

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- Before starting:

Transform BL21(DE3) or BL21(DE3)pLysS cells with the prepared expression plasmid by the standard procedure.

- ‡ Notes for transformation

1. Sometimes, expression may vary among transformants. If large and small colonies are observed in the same plate, the expressed protein may affect the growth of the *E. coli* cells.

2. If the expressed protein is toxic to *E. coli* cells, transformants may not be obtained.

In this case, repression of a basal level expression by T7 promoter may work, see “Notes for expression.”

- Expression:

1. Following transformation, pick a colony and inoculate it into 3 ml of LB medium containing the appropriate antibiotic with shaking at 37°C, overnight. For the BL21(DE3)pLysS strain, it is preferable to add chloramphenicol at a final concentration of 34 µg/ml in the overnight culture to maintain pLysS.

2. The next morning, transfer 0.5 ml of the overnight culture to a new 10 ml of LB medium containing the appropriate antibiotic to select the expression plasmid. Grow the culture with shaking at 37°C until the OD₆₀₀ reaches 0.5 (approximately 2 hrs but this depended on the expression plasmids).

When using BL21(DE3)pLys, chloramphenicol is not usually required in the short-period culture.

3. When the OD₆₀₀ reaches 0.5, transfer an aliquot (e.g., 1 ml) of the culture to a new centrifuge tube and centrifuge it to harvest cells. Store the cells at -80°C until analysis.

Add IPTG to a final concentration of 1 mM to the rest of the culture and grow the culture with shaking at 37°C for 3 hours.

The IPTG concentration and induction time are general values. It is recommended to determine the optimal condition for the target gene expression.

4. After the induction, harvest the cells. To analyze the expression, before harvesting the cells, transfer an aliquot of the culture (e.g., 1 ml) and centrifuge it to precipitate the cells.

- Analysis

1. Suspend the precipitated cells (from the 1 ml culture) in 200 µl of 1× PBS buffer.

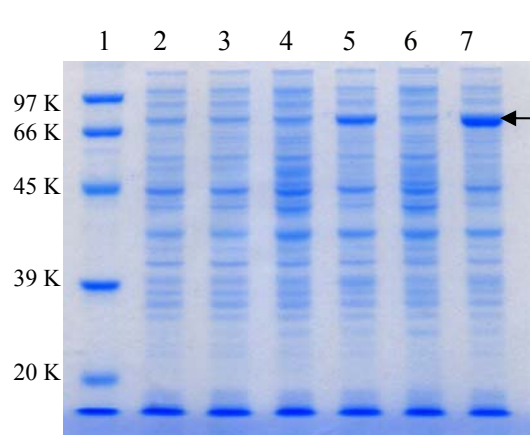
2. Mix an aliquot of the suspension (e.g., 100 µl) with an equal volume of 2 × SDS sample buffer.

3. Heat the mixture at 85°C for 5 min, then centrifuge at 10,000 g for 10 min. Subject the supernatant (e.g., 5-25 µl) to SDS-PAGE. Western blot will help analyzing the expression of the target protein.

- 2 × SDS sample buffer : 2 % sodium dodecyl sulfate, 5 % 2-mercaptoethanol, 20 % glycerol, 0.02 % BPB, 62.5 mM Tris-HCl, pH6.8

- 1× PBS buffer.: 20 mM sodium phosphate, 150 mM sodium chloride, pH7.4

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An arrow shows the expressed 70 KDa proteins. Only induced cells expressed 70 KDa proteins.

Figure of protein expression from pETIA

A gene of 70 KDa protein was cloned into pETIA (pETIA/70K). BL21(DE3) cell was transformed with the pETIA/70K, one of colonies were cultured overnight and transferred to two tubes (#1, #2) containing culture medium. IPTG was added to only tube #2 when the OD₆₀₀ reaches 0.5. At each stage, OD₆₀₀ of the culture was determined and the same amount of cells were lysed and subjected to 10 % polyacrylamide gel SDS electrophoresis.

Lane 1: DynaMarker Protein Eco (#DM610)

Lane 2, 3 : Cells from tubes #1 and 2 before induction.

Lane 4 : Cells (tubes #1), two hours after OD0.5.

Lane 5 : Cells (tubes #2), two hours after induction

Lane 6 : Cells (tubes #1), 4 hours after OD0.5.

Lane 7 : Cells (tubes #2), 4 hours after induction

‡ Notes for expression:

1. As the T7 expression method is a high-level protein expression system, some basal level expression of the target protein will occur in uninduced cells. This is likely problematic in cases in which the target protein is toxic to *E. coli* cells. In this case, it may be necessary to decrease the basal level expression as follows:

- Use a lower-copy number T7 expression vector, pETBA, pETBK, but not pETUA, pETUK
- Use a stringent regulated expression vector, pETIA, pETIK.
- Use liquid medium and agar plates supplemented with glucose (0.5 -1 %).
Glucose is known to decrease a basal expression from *lacUV5* promoter².
- Use BL21(DE3)pLysS strain but not BL21(DE3) strain.

The T7 Lysozyme encoded in a pLysS plasmid reduces the basal level of T7 RNA polymerase Expression³. This leads to suppression of the basal level expression of the target protein.

2. When expressing proteins in BL21(DE3) cells, if it takes longer time (5 hrs or more) to reach 0.5 at OD₆₀₀ after inoculating the overnight culture (0.5 ml) to a new LB medium (10 ml), the expressed protein is likely toxic to *E. coli* cells.

3. When BL21(DE3) cells lyse after induction with IPTG, the expressed protein is likely toxic to *E. coli* cells.

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Reference:

- 1) Studier, F.W. and Moffatt, B.A., *J. Mol. Biol.* 189 (1986) 113–130.
- 2) Pan, S. and Malcom, B.A., *BioTechniques* 29 (2000), 1234–1238
- 3) Moffatt, B.A. and Studier, F.W., *Cell* 49 (1987) 221-227

General reference in this Product Information

Sambrook, J. and Russell, D.W. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Related Products:

DV210	Expression Vector pETBA	DV215	Expression Vector pETIA
DV220	Expression Vector pETUK	DV230	Expression Vector pETBK
DV235	Expression Vector pETIK	DS110	DNA Ligation Kit ver. 2
DS210	Competent Cell JM109	DS220	Competent Cell DH5 α
DS225	Jet Competent Cell (DH5 α)	DS240	Competent Cell BL21
DS255	Zip Competent Cell BL21(DE3)	DS260	Competent Cell BL21(DE3)pLysS
DS500	QuickBlue Protein Staining Solution		

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