

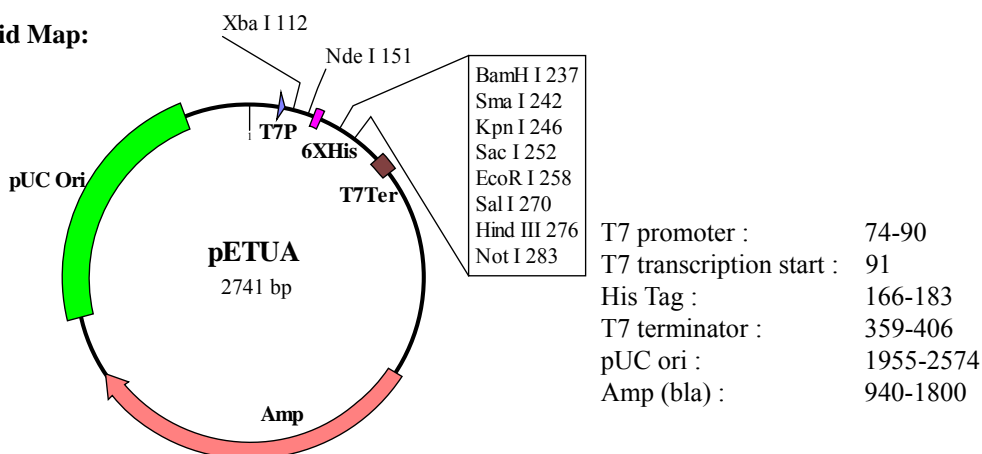
## PRODUCT INFORMATION

### GENERAL INFORMATION

**Product Name :** Expression Vector pETUA  
**Code No. :** DV200  
**Size :** 15 µg (lyophilized plasmid contains salt of TE buffer)  
**Storage :** This product is shipped at ambient temperature. Upon receipt, store at - 20 °C  
**Reconstitution :** Resuspend the lyophilized pETUA with 15 µl of sterile water to make 1 µg/µl plasmid in 1 × TE buffer. After reconstitution, store at - 20 °C

**Product Description :** pETUA is a high copy number, ampicillin resistant, T7 bacterial expression vector. The T7 expression system is one of the strongest expression systems and has been widely used with a coupling of BL21 (DE3) *E. coli* strain. T7 RNA polymerase gene is integrated in a genome of BL21(DE3) under control of lacUV5 promoter. Upon addition of isopropyl-1-thio-β-D-galactopyranoside (IPTG), T7 RNA polymerase is expressed in the BL21(DE3) cells harboring pETUA vector, and it induces a high-level protein expression from T7 promoter of pETUA. BioDynamics Laboratory Inc. offers several kinds of T7 bacterial expression vectors. Among them, pETUA is suitable for a high level expression of non-toxic proteins and the high copy number of pETUA in *E. coli* cells is beneficial for plasmid preparation.

#### Plasmid Map:



#### Features of T7 expression vectors

BioDynamics Laboratory Inc. provides 6 kinds of T7 expression vectors, pETUA, pETBA, pETIA, pETUK, pETBK, and pETIK. These vectors have the same multicloning site and specific feature of each vector is below:

|       | Plasmid copy number | Replicon | Antibiotic resistance | Feature and recommendation              |
|-------|---------------------|----------|-----------------------|---|
| pETUA | high copy           | pUC      | ampicillin            | for non-toxic protein expression        |
| pETBA | medium copy         | pMB1     | ampicillin            | general expression                      |
| pETIA | medium copy         | pMB1     | ampicillin            | stringent regulation with lac repressor |
| pETUK | high copy           | pUC      | kanamycin             | for non-toxic protein expression        |
| pETBK | medium copy         | pMB1     | kanamycin             | general expression                      |
| pETIK | medium copy         | pMB1     | kanamycin             | stringent regulation with lac repressor |

## PRODUCT INFORMATION

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### pETUA Sequence

|             |             |            |            |            |            |      |
|-------------|-------------|------------|------------|------------|------------|------|
| GCGCCCAATA  | CGCAAACCGC  | CTCTCCCCGC | GCGTTGGCCG | ATTCATTAAT | GCAGGATCTC | 60   |
| GATCCCAGCA  | AATTAATACG  | ACTCACTATA | GGGAGACCAC | AACGGTTTCC | CTCTAGAAAT | 120  |
| AATTTTGT    | AACTTTAAGA  | AGGAGATATA | CATATGCGGG | GTTCTCATCA | TCATCATCAT | 180  |
| CATGGTATGG  | CTAGCATGAC  | TGGTGGACAG | CAAATGGGTC | GGGACGATGA | CGATAAGGAT | 240  |
| CCCCGGGTAC  | CGAGCTCGAA  | TTCGATTTTC | TCGACAAGCT | TAGCGGCCGC | CGTTTAATCC | 300  |
| GGCTGCTAAC  | AAAAGCCGAA  | AGGAAGCTGA | GTTGGCTGCT | GCCACCGCTG | AGCAATAACT | 360  |
| AGCATAACCC  | CTTGGGGCCT  | CTAAACGGGT | CTTGAGGGGT | TTTTTGCTGA | AAGGAGGAAC | 420  |
| TATATCCGGA  | TCTGGCGTAA  | TAGCGAAGAG | GCCCCGACCG | ATCGCCCTTC | CCAACAGTTG | 480  |
| CGCAGCCTGA  | ATGGCGAATG  | GCGCCTGATG | CGGTATTTTC | TCCTTACGCA | TCTGTGCGGT | 540  |
| ATTTTCACACC | GCATCTGGTG  | CACTCTCAGT | ACAATCTGCT | CTGATGCCGC | ATAGTTAAGC | 600  |
| CAGCCCCGAC  | ACCCGCCAAC  | ACCCGCTGAC | GCGCCCTGAC | GGGCTTGTCT | GCTCCCGGCA | 660  |
| TCCGCTTACA  | GACAAAGCTGT | GACCGTCTCC | GGGAGCTGCA | TGTGTCAGAG | GTTTTACCG  | 720  |
| TCATCACCGA  | AACGCGCGAG  | ACGAAAGGGC | CTCGTGATAC | GCCTATTTTT | ATAGGTTAAT | 780  |
| GTCATGATAA  | TAATGGTTTC  | TTAGACGTCA | GGTGGCACTT | TTCGGGGAAA | TGTGCGCGGA | 840  |
| ACCCCTATTT  | GTTTATTTTT  | CTAAATACAT | TCAAATATGT | ATCCGCTCAT | GAGACAATAA | 900  |
| CCCTGATAAA  | TGCTTCAATA  | ATATTGAAAA | AGGAAGAGTA | TGAGTATTCA | ACATTTCCGT | 960  |
| GTCGCCCTTA  | TTCCCTTTTT  | TGCGGCATTT | TGCCCTTCTG | TTTTTGCTCA | CCCAGAAACG | 1020 |
| CTGGTGAAAG  | TAAAAGATGC  | TGAAGATCAG | TTGGGTGCAC | GAGTGGGTTA | CATCGAACTG | 1080 |
| GATCTCAACA  | GCGGTAAGAT  | CCTTGAGAGT | TTTCGCCCCG | AAGAACGTTT | TCCAATGATG | 1140 |
| AGCACTTTTA  | AAGTCTGCT   | ATGTGGCGCG | GTATTATCCC | GTATTGACGC | CGGGCAAGAG | 1200 |
| CAACTCGGTC  | GCCGCATACA  | CTATTCTCAG | AATGACTTGG | TTGAGTACTC | ACCAGTCACA | 1260 |
| GAAAAGCATC  | TTACGGATGG  | CATGACAGTA | AGAGAATTAT | GCAGTGCTGC | CATAACCATG | 1320 |
| AGTGATAACA  | CTGCGGCCAA  | CTTACTTCTG | ACAACGATCG | GAGGACCGAA | GGAGCTAACC | 1380 |
| GCTTTTTTGC  | ACAACATGGG  | GGATCATGTA | ACTCGCCTTG | ATCGTTGGGA | ACCGGAGCTG | 1440 |
| AATGAAGCCA  | TACCAAACGA  | CGAGCGTGAC | ACCACGATGC | CTGTAGCAAT | GGCAACAACG | 1500 |
| TTGCGCAAAC  | TATTAACCTGG | CGAACTACTT | ACTCTAGCTT | CCCGGCAACA | ATTAATAGAC | 1560 |
| TGGATGGAGG  | CGGATAAAGT  | TGCAGGACCA | CTTCTGCGCT | CGGCCCTTCC | GGCTGGCTGG | 1620 |
| TTTTATTGCTG | ATAAATCTGG  | AGCCGGTGAG | CGTGGGTCTC | GCGGTATCAT | TGCAGCACTG | 1680 |
| GGGCCAGATG  | GTAAGCCCTC  | CCGTATCGTA | GTTATCTACA | CGACGGGGAG | TCAGGCAACT | 1740 |
| ATGGATGAAC  | GAAATAGACA  | GATCGCTGAG | ATAGGTGCCT | CACTGATTA  | GCATTGGTAA | 1800 |
| CTGTCAGACC  | AAGTTTACTC  | ATATATACTT | TAGATTGATT | TAAAACCTCA | TTTTTAATTT | 1860 |
| AAAAGGATCT  | AGGTGAAGAT  | CCTTTTTGAT | AATCTCATGA | CCAAAATCCC | TTAACGTGAG | 1920 |
| TTTTTCGTTCC | ACTGAGCGTC  | AGACCCCGTA | GAAAAGATCA | AAGGATCTTC | TTGAGATCCT | 1980 |
| TTTTTTCTGC  | GCGTAATCTG  | CTGCTTGCAA | ACAAAAAAC  | CACCGCTACC | AGCGGTGGTT | 2040 |
| TGTTTGCCCG  | ATCAAGAGCT  | ACCAACTCTT | TTTCCGAAGG | TAAC TGCTT | CAGCAGAGCG | 2100 |
| CAGATACCAA  | ATACTGTTCT  | TCTAGTGTAG | CCGTAGTTAG | GCCACCACTT | CAAGAACTCT | 2160 |
| GTAGCACCGC  | CTACATACCT  | CGCTCTGCTA | ATCCTGTTAC | CAGTGGCTGC | TGCCAGTGGC | 2220 |
| GATAAGTCGT  | GTCTTACCGG  | GTTGGACTCA | AGACGATAGT | TACCGGATAA | GGCGCAGCGG | 2280 |

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|            |             |            |            |            |            |      |
|------------|-------------|------------|------------|------------|------------|------|
| TCGGGCTGAA | CGGGGGGTTTC | GTGCACACAG | CCCAGCTTGG | AGCGAACGAC | CTACACCGAA | 2340 |
| CTGAGATACC | TACAGCGTGA  | GCTATGAGAA | AGCGCCACGC | TTCCCGAAGG | GAGAAAGGCG | 2400 |
| GACAGGTATC | CGGTAAGCGG  | CAGGGTCGGA | ACAGGAGAGC | GCACGAGGGA | GCTTCCAGGG | 2460 |
| GGAAACGCCT | GGTATCTTTA  | TAGTCCTGTC | GGGTTTCGCC | ACCTCTGACT | TGAGCGTCGA | 2520 |
| TTTTTGTGAT | GCTCGTCAGG  | GGGGCGGAGC | CTATGGAAAA | ACGCCAGCAA | CGCGGCCTTT | 2580 |
| TTACGGTTCC | TGGCCTTTTG  | CTGGCCTTTT | GCTCACATGT | TCTTTCCTGC | GTTATCCCCT | 2640 |
| GATTCTGTGG | ATAACCGTAT  | TACCGCCTTT | GAGTGAGCTG | ATACCGCTCG | CCGCAGCCGA | 2700 |
| ACGACCGAGC | GCAGCGAGTC  | AGTGAGCGAG | GAAGCGGAAG | A          |            | 2741 |

## PRODUCT INFORMATION

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### PRODUCT USAGE

#### Cloning of a gene to pETUA:

Below is the multiple cloning site of pETUA. To express a recombinant protein correctly, it is necessary to clone the gene of interest in frame with an N-terminal peptide of pETUA. The start codon of pETUA is boxed ATG in the below figure. Digest pETUA 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 pETUA 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 $\alpha$  or JM109. Recombinant plasmids derived from pETUA 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
GATCCC GCGA AATTAATACG ACTCACTATA GGGAGACCAC AACGGTTTCC CTCTAGAAAT 120
AspProAlaL ysLeuIleAr gLeuThrIle GlyArgProG lnArgPhePr oSerArgAsn
                NdeI                                6xHis
AATTTTGT TTT AACTTTAAGA AGGAGATATA CATATGCGGG GTTCTCATCA TCATCATCAT 180
AsnPheVal* **Leu***Gl uGlyAspIle HisMetArgG lySerHisHi sHisHisHis
                EK                                BamHI
CATGGTATGG CTAGCATGAC TGGTGGACAG CAAATGGGTC GGGACGATGA CGATAAGGAT 240
HisGlyMetA laSerMetTh rGlyGlyGln GlnMetGlyA rgAspAspAs pAspLysAsp
                KpnI                                EcoRI                                SalI                                NotI                                ↑
CCCCGGGTAC CGAGCTCGAA TTCGATTTTCG TCGACAAGCT TAGCGGCCGC CGTTTAAATCC 300
                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

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### 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.

#### • 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 OD<sub>600</sub> 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 OD<sub>600</sub> 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.

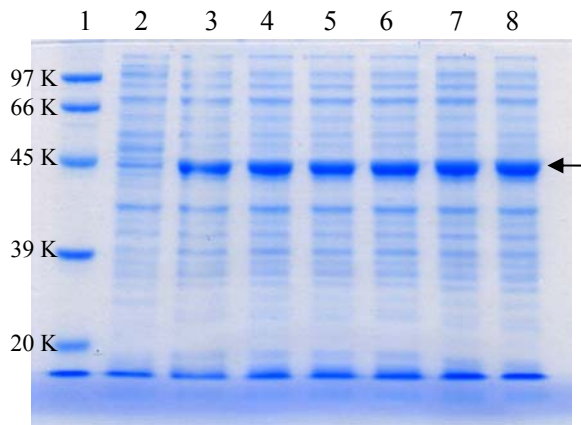
2. Mix an aliquot of the suspension (e.g., 100 µl) with an equal volume of 2 × SDS sample buffer.

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 44 KDa proteins.

Figure of protein expression from pETUA

A gene of 44 KDa protein was cloned into pETUA (pETUA/44K). BL21(DE3) cell was transformed with the pETUA/44K, six colonies were picked and followed the "Protein Expression Procedure" as above. After induction, aliquot of the cells from each culture was subjected to 10 % polyacrylamide gel SDS electrophoresis. The gel was stained with Quick Blue Protein Staining Solution (BioDynamics Laboratory Inc. #DS500). Lane 1: DynaMarker Protein Eco ( #DM610) Lane 2 : BL21(DE3) harboring pETUA but not pETUA/44K Lane 3-8 : BL21(DE3) cells, clones 1-6

### ‡ 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* promoter<sup>2</sup>.
- 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 Expression<sup>3</sup>. This leads to suppression of the basal level expression of the target protein.

2. When expressing proteins in BL21(DE3) cells, if it takes a longer time (5 hrs or more) to reach 0.5 at OD<sub>600</sub> 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 $\alpha$   |
| DS225 | Jet Competent Cell (DH5 $\alpha$ )  | DS240 | Competent Cell BL21           |
| DS255 | Zip Competent Cell BL21(DE3)        | DS260 | Competent Cell BL21(DE3)pLysS |
| DS500 | QuickBlue Protein Staining Solution |       |                               |

### ● Purchaser Notification

This product is manufactured based on the T7 expression system which is the subject of US patent applications assigned to Brookhaven Science Associates, LLC (BSA). The product must be used only outside the United States and its territories. Neither this product nor materials prepared used by the T7 expression system are allowed to be distributed in the US and its territories without license of BSA. Information about license regarding the T7 expression system may be obtained from the Office of Intellectual Property and Sponsored Research, Brookhaven National Laboratory, Building 185, P.O. Box 500, Upton, New York 11973-5000, USA.