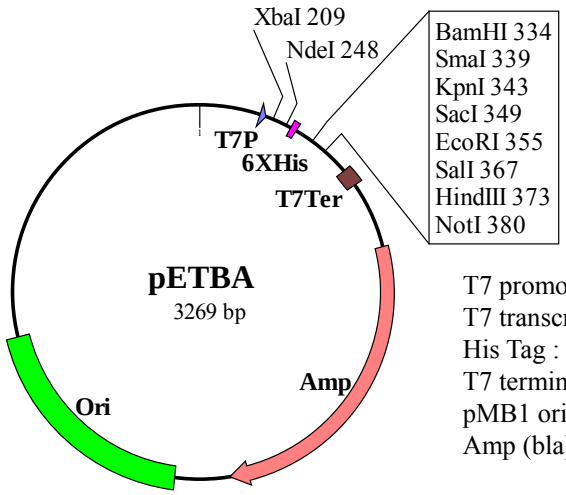
PRODUCT INFORMATION

GENERAL INFORMATION

Product Name : Expression Vector pETBA
Code No. : DV210
Size : 15 µg (lyophilized plasmid contains salt of TE buffer)
Storage : This product is shipped at ambient temperature. Upon receipt, store at - 20 ℃
Reconstitution : Resuspend the lyophilized pETBA with 15 µl of sterile water to make 1 µg/µl plasmid in 1 × TE buffer. After reconstitution, store at - 20 ℃

Product Description : pETBA is a medium 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* cell. 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 pETBA vector, and it induces a high-level protein expression from T7 promoter of pETBA. BioDynamics Laboratory Inc. offers several kinds of T7 bacterial expression vectors. Among them, pETBA is standard vector for a high level expression of proteins.

Plasmid Map:



T7 promoter :	171-187
T7 transcription start :	188
His Tag :	263-280
T7 terminator :	456-503
pMB1 ori :	1706-2325
Amp (bla) :	691-1551

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

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pETBA Sequence

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CAGACGTTTT GCAGCAGCAG TCGCTTCACG TTCGCTCGCG TATCGGTGAT TCATTCTGCT    60
AACCAGTAAG GCAACCCCGC CAGCCTAGCC GGGTCCTCAA CGACAGGAGC ACGATCATGC   120
GCACCCGTGG CCAGGACCCA ACGCTGCCCG AGATCTCGAT CCCGCGAAAT TAATACGACT   180
CACTATAGGG AGACCACAAC GGTTTCCCTC TAGAAATAAT TTTGTTTAAC TTTAAGAAGG   240
AGATATACAT ATGCGGGGTT CTCATCATCA TCATCATCAT GGTATGGCTA GCATGACTGG   300
TGGACAGCAA ATGGGTCGGG ACGATGACGA TAAGGATCCC CGGGTACCGA GCTCGAATTC   360
GATTTCGTCG ACAAGCTTAG CGGCCGCCGT TTAATCCGGC TGCTAACAAA GCCCGAAAGG   420
AAGCTGAGTT GGCTGCTGCC ACCGCTGAGC AATAACTAGC ATAACCCCTT GGGGCCTCTA   480
AACGGGTCTT GAGGGGTTTT TTGCTGAAAG GAGGAACTAT ATCCGGATAA TGTCATGATA   540
ATAATGGTTT CTTAGACGTC AGGTGGCACT TTTCGGGGAA ATGTGCGCGG AACCCCTATT   600
TGTTTATTTT TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA   660
ATGCTTCAAT AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTCGCCCTT   720
ATTCCCTTTT TTGCGGCATT TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA   780
GTAAAAGATG CTGAAGATCA GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC   840
AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT   900
AAAGTTCTGC TATGTGGCGC GGTATTATCC CGTGTTGACG CCGGGCAAGA GCAACTCGGT   960
CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC AGAAAAGCAT  1020
CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC  1080
ACTGCGGCCA ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG  1140
CACAACATGG GGGATCATGT AACTCGCCTT GATCGTTGGG AACCGGAGCT GAATGAAGCC  1200
ATACCAAACG ACGAGCGTGA CACCACGATG CCTGCAGCAA TGGCAACAAC GTTGCGCAAA  1260
CTATTAACTG GCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG  1320
GCGGATAAAG TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT  1380
GATAAATCTG GAGCCGGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT  1440
GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA  1500
CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC  1560
CAAGTTTACT CATATATACT TTAGATTGAT TTAAAACTTC ATTTTTAATT TAAAAGGATC  1620
TAGGTGAAGA TCCTTTTTGA TAATCTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTC  1680
CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTTCTG  1740
CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG  1800
GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAACTGGCT TCAGCAGAGC GCAGATACCA  1860
AATACTGTCC TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG  1920
CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG  1980
TGTCTTACCG GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA  2040
ACGGGGGGTT CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC  2100
CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT  2160
CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC  2220
TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTTGTGA  2280
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TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC  2380
CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG  2400
GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG AACGACCGAG  2460
CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GAGCGCCTGA TGCGGTATTT TCTCCTTACG  2520
CATCTGTGCG GTATTTCACA CCGCATATAT GGTGCACTCT CAGTACAATC TGCTCTGATG  2580
CCGCATAGTT AAGCCAGTAT ACACTCCGCT ATCGCTACGT GACTGGGTCA TGGCTGCGCC  2640
CCGACACCCG CCAACACCCG CTGACGCGCC CTGACGGGCT TGTCTGCTCC CGGCATCCGC  2700
TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC  2760
ACCGAAACGC GCGAGGCAGC TGCGGTAAAG CTCATCAGCG TGGTCGTGAA GCGATTCACA  2820
GATGTCTGCC TGTTCATCCG CGTCCAGCTC GTTGAGTTTC TCCAGAAGCG TTAATGTCTG  2880
GCTTCTGATA AAGCGGGCCA TGTTAAGGGC GGTTTTTTCC TGTTTGGTCA CTGATGCCTC  2940
CGTGTAAGGG GGATTTCTGT TCATGGGGGT AATGATACCG ATGAAACGAG AGAGGATGCT  3000
CACGATACGG GTTACTGATG ATGAACATGC CCGGTTACTG GAACGTTGTG AGGGTAAACA  3060
ACTGGCGGTA TGGATGCGGC GGGACCAGAG AAAAATCACT CAGGGTCAAT GCCAGCGCTT  3120
CGTTAATACA GATGTAGGTG TTCCACAGGG TAGCCAGCAG CATCCTGCGA TGCAGATCCG  3180
GAACATAATG GTGCAGGGCG CTGACTTCCG CGTTTCCAGA CTTTACGAAA CACGGAAACC  3240
GAAGACCATT CATGTTGTTG CTCAGGTCG                                    3269
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PRODUCT INFORMATION

PRODUCT USAGE

Cloning of a gene to pETBA:

Below is the multiple cloning site of pETBA. To express a recombinant protein correctly, it is necessary to clone the gene of interest in frame with an N-terminal peptide of pETBA. The start codon of pETBA is boxed ATG in the below figure. Digest pETBA 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 pETBA 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 DH5α or JM109. Recombinant plasmids derived from pETBA 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.

```
               T7 promoter                                  XbaI
GATCCCGCGA AATTAATACG ACTCACTATA GGGAGACCAC AACGGTTTCC CTCTAGAAAT   217
AspProAlaL ysLeuIleAr gLeuThrIle GlyArgProG lnArgPhePr oSerArgAsn
                                 NdeI              6×His
AATTTTGTTT AACTTTAAGA AGGAGATATA CATATGCGGG GTTCTCATCA TCATCATCAT   277
AsnPheVal* **Leu***Gl uGlyAspIle HisMetArgG lySerHisHi sHisHisHis
                                                    EK     BamHI
CATGGTATGG CTAGCATGAC TGGTGGACAG CAAATGGGTC GGGACGATGA CGATAAGGAT   337
HisGlyMetA laSerMetTh rGlyGlyGln GlnMetGlyA rgAspAspAs pAspLysAsp
     KpnI          EcoRI        SalI            NotI              ↑
CCCCGGGTAC CGAGCTCGAA TTCGATTTCG TCGACAAGCT TAGCGGCCGC CGTTTAATCC   397
  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_{600} 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_{600} 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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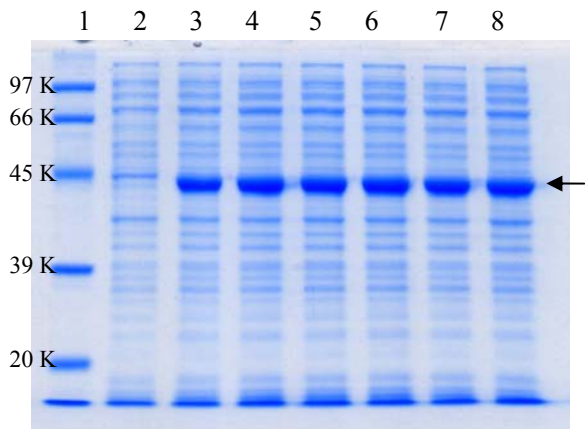

An arrow shows the expressed 44 KDa proteins.

Figure of protein expression from pETUA

A gene of 44 KDa protein was cloned into pETBA (pETBA/44K). BL21(DE3) cell was transformed with the pETBA/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 pETBA but not pETBA/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:

a) Use a lower-copy number T7 expression vector, pETBA, pETBK, but not pETUA, pETUK

b) Use a stringent regulated expression vector, pETIA, pETIK.

c) Use liquid medium and agar plates supplemented with glucose (0.5 -1 %).
Glucose is known to decrease a basal expression from *lac*UV5 promoter[2].

d) 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[3]. 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_{600} 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 DH5α
DS225	Jet Competent Cell (DH5α)	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.