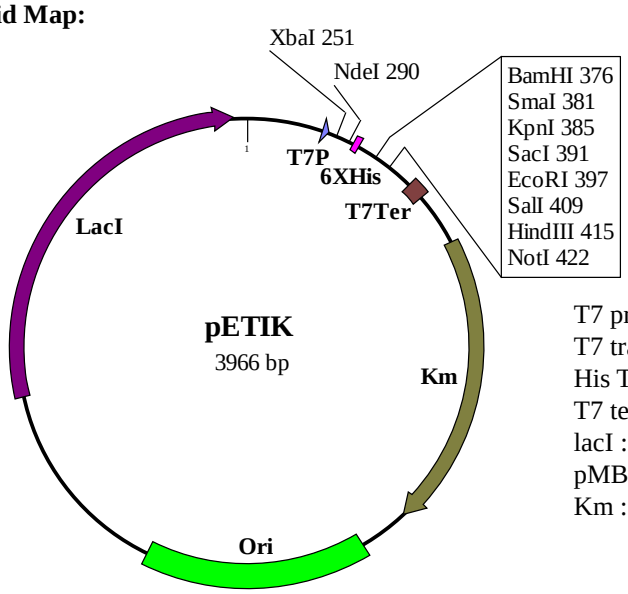
PRODUCT INFORMATION

GENERAL INFORMATION

Product Name : pET Expression Vector pETIK
Code No. : DV235
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 pETIK with 15 µl of sterile water to make 1 µg/µl plasmid in 1 × TE buffer. After reconstitution, store at - 20 ℃

Product Description : pETIK is a medium copy number, kanamycin resistant, stringent controllable 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 pETIK vector, and it induces a high-level protein expression from T7 promoter of pETIK. The pETIK has a lacI gene, which represses T7 RNA polymerase gene in the absence of IPTG. The regulation with lac repressor is beneficial to repress a basal level protein expression and to maintain a recombinant plasmid in BL21 (DE3) cell.

Plasmid Map:



T7 promoter :	213-229
T7 transcription start :	230
His Tag :	305-322
T7 terminator :	498-545
lacI :	2845-3927
pMB1 ori :	1649-2268
Km :	692-1507

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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

pETIK Sequence

```
GTTTGACAGC TTATCATCGA CTGCACGGTG CACCAATGCT TCTGGCGTCA GGCAGCCATC    60
GGAAGCTGTG GTATGGCTGT GCAGGTCGTA AATCACTGCA TAATTCGTGT CGCTCAAGGC   120
GCACTCCCGT TCTGGATAAT GTTTTTTGCG CCGACATCAT AACGGTTCTG GCAAATATTC   180
TGAAATGAGC TGAGATCTCG ATCCCGCGAA ATTAATACGA CTCACTATAG GGAGACCACA   240
ACGGTTTCCC TCTAGAAATA ATTTTGTTTA ACTTTAAGAA GGAGATATAC ATATGCGGGG   300
TTCTCATCAT CATCATCATC ATGGTATGGC TAGCATGACT GGTGGACAGC AAATGGGTCG   360
GGACGATGAC GATAAGGATC CCCGGGTACC GAGCTCGAAT TCGATTTCGT CGACAAGCTT   420
AGCGGCCGCC GTTTAATCCG GCTGCTAACA AAGCCCGAAA GGAAGCTGAG TTGGCTGCTG   480
CCACCGCTGA GCAATAACTA GCATAACCCC TTGGGGCCTC TAAACGGGTC TTGAGGGGTT   540
TTTTGCTGAA AGGAGGAACT ATATCCGGAT GCGTTTCTAC AAACTCTTTT GTTTATTTTT   600
CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTATGTC   660
TGCTTACATA AACAGTAATA CAAGGGGTGT TATGAGCCAT ATTCAACGGG AAACGTCTTG   720
CTCAAGGCCG CGATTAAATT CCAACATGGA TGCTGATTTA TATGGGTATA AATGGGCTCG   780
CGATAATGTC GGGCAATCAG GTGCGACAAT CTATCGATTG TATGGGAAGC CCGATGCGCC   840
AGAGTTGTTT CTGAAACATG GCAAAGGTAG CGTTGCCAAT GATGTTACAG ATGAGATGGT   900
CAGACTAAAC TGGCTGACGG AATTTATGCC TCTTCCGACC ATCAAGCATT TTATCCGTAC   960
TCCTGATGAT GCATGGTTAC TCACCACTGC GATCCCAGGG AAAACAGCAT TCCAGGTATT  1020
AGAAGAATAT CCTGATTCAG GTGAAAATAT TGTTGATGCG CTGGCAGTGT TCCTGCGCCG  1080
GTTGCATTCG ATTCCTGTTT GTAATTGTCC TTTTAACAGC GATCGCGTAT TTCGTCTCGC  1140
TCAGGCGCAA TCACGAATGA ATAACGGTTT GGTTGATGCG AGTGATTTTG ATGACGAGCG  1200
TAATGGCTGG CCTGTTGAAC AAGTCTGGAA AGAAATGCAT AAGCTATTGC CATTCTCACC  1260
GGATTCAGTC GTCACTCATG GTGATTTCTC ACTTGATAAC CTTATTTTTG ACGAGGGGAA  1320
ATTAATAGGT TGTATTGATG TTGGACGAGT CGGAATCGCA GACCGATACC AGGATCTTGC  1380
CATCCTATGG AACTGCCTCG GTGAGTTTTC TCCTTCATTA CAGAAACGGC TTTTTCAAAA  1440
ATATGGTATT GATAATCCTG ATATGAATAA ATTGCAGTTT CATTTGATGC TCGATGAGTT  1500
TTTCTAATTA AAACATATAT ACTTTAGATT GATTTAAAAC TTCATTTTTA ATTTAAAAGG  1560
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ATCTAGGTGA AGATCCTTTT TGATAATCTC ATGACCAAAA TCCCTTAACG TGAGTTTTCG  1620
TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAGGAT CTTCTTGAGA TCCTTTTTTT  1680
CTGCGCGTAA TCTGCTGCTT GCAAACAAAA AAACCACCGC TACCAGCGGT GGTTTGTTTG  1740
CCGGATCAAG AGCTACCAAC TCTTTTTCCG AAGGTAACTG GCTTCAGCAG AGCGCAGATA  1800
CCAAATACTG TCCTTCTAGT GTAGCCGTAG TTAGGCCACC ACTTCAAGAA CTCTGTAGCA  1860
CCGCCTACAT ACCTCGCTCT GCTAATCCTG TTACCAGTGG CTGCTGCCAG TGGCGATAAG  1920
TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG ATAAGGCGCA GCGGTCGGGC  1980
TGAACGGGGG GTTCGTGCAC ACAGCCCAGC TTGGAGCGAA CGACCTACAC CGAACTGAGA  2040
TACCTACAGC GTGAGCTATG AGAAAGCGCC ACGCTTCCCG AAGGGAGAAA GGCGGACAGG  2100
TATCCGGTAA GCGGCAGGGT CGGAACAGGA GAGCGCACGA GGGAGCTTCC AGGGGGAAAC  2160
GCCTGGTATC TTTATAGTCC TGTCGGGTTT CGCCACCTCT GACTTGAGCG TCGATTTTTG  2220
TGATGCTCGT CAGGGGGGCG GAGCCTATGG AAAAACGCCA GCAACGCGGC CTTTTTACGG  2280
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GTGGATAACC GTATTACCGC CTTTGAGTGA GCTGATACCG CTCGCCGCAG CCGAACGACC  2400
GAGCGCAGCG AGTCAGTGAG CGAGGAAGCG GAAGAGCGCC TGATGCGGTA TTTTCTCCTT  2460
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CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG TGTCAGAGGT TTTCACCGTC  2700
ATCACCGAAA CGCGCGAGGC AGCAGATCAA TTCGCGCGCG AAGGCGAAGC GGCATGCATT  2760
TACGTTGACA CCATCGAATG GTGCAAAACC TTTCGCGGTA TGGCATGATA GCGCCCGGAA  2820
GAGAGTCAAT TCAGGGTGGT GAATGTGAAA CCAGTAACGT TATACGATGT CGCAGAGTAT  2880
GCCGGTGTCT CTTATCAGAC CGTTTCCCGC GTGGTGAACC AGGCCAGCCA CGTTTCTGCG  2940
AAAACGCGGG AAAAAGTGGA AGCGGCGATG GCGGAGCTGA ATTACATTCC CAACCGCGTG  3000
GCACAACAAC TGGCGGGCAA ACAGTCGTTG CTGATTGGCG TTGCCACCTC CAGTCTGGCC  3060
CTGCACGCGC CGTCGCAAAT TGTCGCGGCG ATTAAATCTC GCGCCGATCA ACTGGGTGCC  3120
AGCGTGGTGG TGTCGATGGT AGAACGAAGC GGCGTCGAAG CCTGTAAAGC GGCGGTGCAC  3180
AATCTTCTCG CGCAACGCGT CAGTGGGCTG ATCATTAACT ATCCGCTGGA TGACCAGGAT  3240
GCCATTGCTG TGGAAGCTGC CTGCACTAAT GTTCCGGCGT TATTTCTTGA TGTCTCTGAC  3300
CAGACACCCA TCAACAGTAT TATTTTCTCC CATGAAGACG GTACGCGACT GGGCGTGGAG  3360
CATCTGGTCG CATTGGGTCA CCAGCAAATC GCGCTGTTAG CGGGCCCATT AAGTTCTGTC  3420
TCGGCGCGTC TGCGTCTGGC TGGCTGGCAT AAATATCTCA CTCGCAATCA AATTCAGCCG  3480
ATAGCGGAAC GGGAAGGCGA CTGGAGTGCC ATGTCCGGTT TTCAACAAAC CATGCAAATG  3540
CTGAATGAGG GCATCGTTCC CACTGCGATG CTGGTTGCCA ACGATCAGAT GGCGCTGGGC  3600
GCAATGCGCG CCATTACCGA GTCCGGGCTG CGCGTTGGTG CGGATATCTC GGTAGTGGGA  3660
TACGACGATA CCGAAGACAG CTCATGTTAT ATCCCGCCGT CAACCACCAT CAAACAGGAT  3720
TTTCGCCTGC TGGGGCAAAC CAGCGTGGAC CGCTTGCTGC AACTCTCTCA GGGCCAGGCG  3780
GTGAAGGGCA ATCAGCTGTT GCCCGTCTCA CTGGTGAAAA GAAAAACCAC CCTGGCGCCC  3840
AATACGCAAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATGCAGCT GGCACGACAG  3900
GTTTCCCGAC TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT AGCGCGAATT  3960
GATCTG                                                            3966
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PRODUCT USAGE

Cloning of a gene to pETIK:

Below is the multiple cloning site of pETIK. To express a recombinant protein correctly, it is necessary to clone the gene of interest in frame with an N-terminal peptide of pETIK. The start codon of pETIK is boxed ATG in the below figure. Digest pETIK 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 pETIK 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. In the transformation, recombinant cells should be selected on LB agar plates containing 15-25 μg/ml of kanamycin, because higher concentration of kanamycin often retarded cell growth on the agar plates. Recombinant plasmids derived from pETIK 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   259
AspProAlaL ysLeuIleAr gLeuThrIle GlyArgProG lnArgPhePr oSerArgAsn
                                  NdeI              6×His
AATTTTGTTT 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 TTCGATTTCG TCGACAAGCT TAGCGGCCGC CGTTTAATCC   439
  SmaI        SacI                     HindIII
ProArgValP roSerSerAs nSerIleSer SerThrSerL euAlaAlaAl aVal***Ser
```

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. If kanamycin is used for selection, recombinant cells should be selected on LB agar plates containing 15-25 µg/ml of kanamycin, because a higher concentration of kanamycin often retards cell growth on the agar plates.

‡ 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 (kanamycin is often used at 25-30 µg/ml for liquid culture) 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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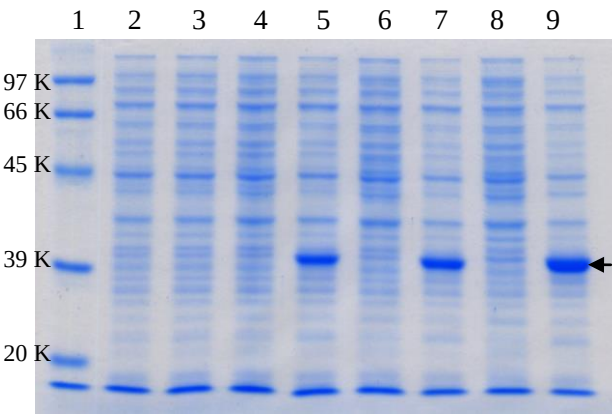

Figure of protein expression from pETIK

A gene of 40 KDa protein was cloned into pETIK (pETIK/40K). BL21(DE3) cell was transformed with the pETIK/40K, one of colonies was cultured overnight and transferred to two tubes (#1, #2) containing culture medium. IPTG was added to only tube #2 when the OD_{600} reached 0.5. At each stage, OD_{600} of the culture was determined and the same amount of cells were lysed and subjected to 10 % polyacrylamide gel SDS electrophoresis.

An arrow shows the expressed 40 KDa protein. Only inducted cells expressed 40 KDa proteins.

Lane 1: DynaMarker Protein Eco (#DM610)
Lane 2, 3 : Cells from tubes #1 and 2 before induction.
Lane 4 : Cells (tube #1), 1 hour after OD600 reached 0.5
Lane 5 : Cells (tube #2), 1 hour after induction
Lane 6 : Cells (tube #1), 2 hours after OD600 reached 0.5
Lane 7 : Cells (tube #2), 2 hours after induction
Lane 8 : Cells (tube #1), 4 hours after OD600 reached 0.5
Lane 9 : Cells (tube #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:

   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 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	pET Expression Vector pETBA	DV215	pET Expression Vector pETIA
DV220	pET Expression Vector pETUK	DV230	pET Expression Vector pETBK
DV235	pET 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.

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