

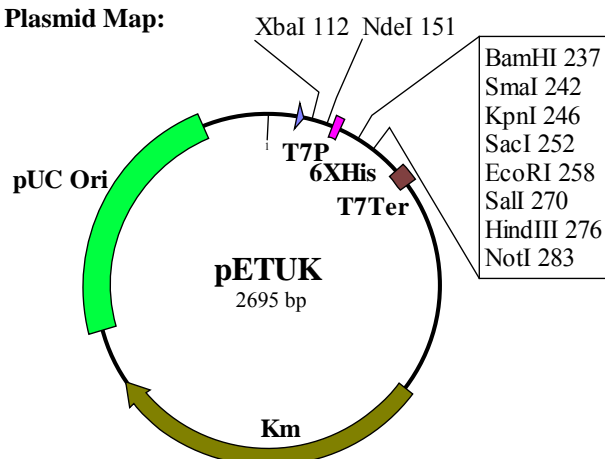
## PRODUCT INFORMATION

### GENERAL INFORMATION

**Product Name :** Expression Vector pETUK  
**Code No. :** DV220  
**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 pETUK with 15 µl of sterile water to make 1 µg/µl plasmid in 1 × TE buffer. After reconstitution, store at - 20 °C

**Product Description :** pETUK is a high copy number, kanamycin 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 pETUK vector, and it induces a high-level protein expression from T7 promoter of pETUK. BioDynamics Laboratory Inc. offers several kinds of T7 bacterial expression vectors. Among them, pETUK is suitable for a high level expression of non-toxic proteins and the high copy number of pETUK in *E. coli* cells is beneficial for plasmid preparation.

#### Plasmid Map:



T7 promoter :	74-90
T7 transcription start :	91
His Tag :	166-183
T7 terminator :	359-406
pUC ori :	1909-2528
Km :	952-1767

#### 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

### pETUK Sequence

GCGCCCAATA	CGCAAACCGC	CTCTCCCCGC	GCGTTGGCCG	ATTCATTAAT	GCAGGATCTC	60
GATCCCGCGA	AATTAATACG	ACTCACTATA	GGGAGACCAC	AACGGTTTCC	CTCTAGAAAT	120
AATTTTGTTF	AACTTTAAGA	AGGAGATATA	CATATGCGGG	GTTCTCATCA	TCATCATCAT	180
CATGGTATGG	CTAGCATGAC	TGGTGGACAG	CAAATGGGTC	GGGACGATGA	CGATAAGGAT	240
CCCCGGGTAC	CGAGCTCGAA	TTCGATTTTC	TCGACAAGCT	TAGCGGCCGC	CGTTTAATCC	300
GGCTGCTAAC	AAAGCCCGAA	AGGAAGCTGA	GTTGGCTGCT	GCCACCGCTG	AGCAATAACT	360
AGCATAACCC	CTTGGGGCCT	CTAAACGGGT	CTTGAGGGGT	TTTTTGCTGA	AAGGAGGAAC	420
TATATCCGGA	TCTGGCGTAA	TAGCGAAGAG	GCCCCACCG	ATCGCCCTTC	CCAACAGTTG	480
CGCAGCCTGA	ATGGCGAATG	GCGCCTGATG	CGGTATTTTC	TCCTTACGCA	TCTGTGCGGT	540
ATTTTCACACC	GCATCTGGTG	CACTCTCAGT	ACAATCTGCT	CTGATGCCGC	ATAGTTAAGC	600
CAGCCCCGAC	ACCCGCCAAC	ACCCGCTGAC	GCGCCCTGAC	GGGCTTGTCT	GCTCCC GGCA	660
TCCGCTTACA	GACAAGCTGT	GACCGTCTCC	GGGAGCTGCA	TGTGTCAGAG	GTTTTACCG	720
TCATCACCGA	AACGCGCGAG	ACGAAAGGGC	CTCGTGATAC	GCCTATTTTT	ATAGTTAAT	780
GTCATGATAA	TAATGGTTTC	TTAGACGTCA	GTTGGCACCT	TTCGGGGAAA	TGTGCGCGGA	840
ACCCCTATTT	GTTTATTTTT	CTAAATACAT	TCAAATATGT	ATCCGCTCAT	GAGACAATAA	900
CCCTGATAAA	TGCTTATGTC	TGCTTACATA	AACAGTAATA	CAAGGGGTGT	TATGAGCCAT	960
ATTCAACGGG	AAACGTCCTG	CTCAAGGCCG	CGATTAAATT	CCAACATGGA	TGCTGATTTA	1020
TATGGGTATA	AATGGGCTCG	CGATAATGTC	GGGCAATCAG	GTGCGACAAT	CTATCGATTG	1080
TATGGGAAGC	CCGATGCGCC	AGAGTTGTTT	CTGAAACATG	GCAAAGGTAG	CGTTGCCAAT	1140
GATGTTACAG	ATGAGATGGT	CAGACTAAAC	TGGCTGACGG	AATTTATGCC	TCTTCCGACC	1200
ATCAAGCATT	TTATCCGTAC	TCCTGATGAT	GCATGGTTAC	TCACCCTGTC	GATCCCAGGG	1260
AAAAACAGCAT	TCCAGGTATT	AGAAGAATAT	CCTGATTCAG	GTGAAAATAT	TGTTGATGCG	1320
CTGGCAGTGT	TCCTGCGCCG	GTTGCATTTC	ATTCCCTGTT	GTAATTGTCC	TTTTAACAGC	1380
GATCGCGTAT	TTCGTCTCGC	TCAGGCGCAA	TCACGAATGA	ATAACGGTTT	GGTTGATGCG	1440
AGTGATTTTG	ATGACGAGCG	TAATGGCTGG	CCTGTTGAAC	AAGTCTGGAA	AGAAATGCAT	1500
AAGCTATTGC	CATTCTCACC	GGATTCAATC	GTCACCTCAT	GTGATTTCTC	ACTTGATAAC	1560
CTTATTTTTT	ACGAGGGGAA	ATTAATAGGT	TGTATTGATG	TTGGACGAGT	CGGAATCGCA	1620
GACCGATAAC	AGGATCTTGC	CATCCTATGG	AACTGCCTCG	GTGAGTTTTT	TCCTTCATTA	1680
CAGAAACGGC	TTTTTCAAAA	ATATGGTATT	GATAATCCTG	ATATGAATAA	ATTGCAGTTT	1740
CATTTGATGC	TCGATGAGTT	TTTCTAATTA	AAACATATAT	ACTTTAGATT	GATTTAAAAC	1800
TTCATTTTTA	ATTTAAAAGG	ATCTAGGTGA	AGATCCTTTT	TGATAATCTC	ATGACCAAAA	1860
TCCCTTAACG	TGAGTTTTTC	TTCCACTGAG	CGTCAGACCC	CGTAGAAAAG	ATCAAAGGAT	1920
CTTCTTGAGA	TCCTTTTTTT	CTGCGCGTAA	TCTGCTGCTT	GCAAACAAAA	AAACCACCGC	1980
TACCAGCGGT	GGTTTGTFTG	CCGGATCAAG	AGCTACCAAC	TC'TTTTTCCG	AAGGTAAC'TG	2040
GCTTCAGCAG	AGCGCAGATA	CCAAATACTG	TTCTTCTAGT	GTAGCCGTAG	TTAGGCCACC	2100
ACTTCAAGAA	CTCTGTAGCA	CCGCCTACAT	ACCTCGCTCT	GCTAATCCTG	TTACCAGTGG	2160
CTGCTGCCAG	TGGCGATAAG	TCGTGTCTTA	CCGGTTTGGG	CTCAAGACGA	TAGTTACCGG	2220
ATAAGGCGCA	GCGGTCGGGC	TGAACGGGGG	GTTTCGTGCAC	ACAGCCCAGC	TTGGAGCGAA	2280
CGACCTACAC	CGAACTGAGA	TACCTACAGC	GTGAGCTATG	AGAAAGCGCC	ACGCTTCCCG	2340
AAGGGAGAAA	GGCGGACAGG	TATCCGGTAA	GCGGCAGGGT	CGGAACAGGA	GAGCGCACGA	2400
GGGAGCTTCC	AGGGGGAAAC	GCCTGGTATC	TTTATAGTCC	TGTCGGGTTT	CGCCACCTCT	2460
GACTTGAGCG	TCGATTTTTG	TGATGCTCGT	CAGGGGGGCG	GAGCCTATGG	AAAAACGCCA	2520
GCAACGCGGC	CTTTTTACGG	TTCTTGGCCT	TTTGCTGGCC	TTTTGCTCAC	ATGTTCTTTC	2580
CTGCGTTATC	CCCTGATTCT	GTGGATAACC	GTATTACCGC	CTTTGAGTGA	GCTGATAACCG	2640
CTCGCCGACG	CCGAACGACC	GAGCGCAGCG	AGTCACTGAG	CGAGGAAGCG	GAAGA	2695

## PRODUCT INFORMATION

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### PRODUCT USAGE

#### Cloning of a gene to pETUK:

Below is the multiple cloning site of pETUK. To express a recombinant protein correctly, it is necessary to clone the gene of interest in frame with an N-terminal peptide of pETUK. The start codon of pETUK is boxed ATG in the below figure. Digest pETUK 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 pETUK 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. In the transformation, recombinant cells should be selected on LB agar plates containing 15-25  $\mu$ g/ml of kanamycin., because higher concentration of kanamycin often retarded cell growth on the agar plates. Recombinant plasmids derived from pETUK 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 120
AspProAlaL ysLeuIleAr gLeuThrIle GlyArgProG lnArgPhePr oSerArgAsn
                NdeI                                6xHis
AATTTTGTTT 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. 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<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.

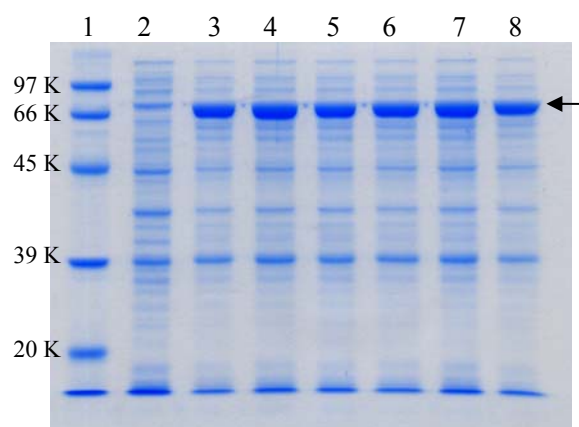
2. Mix an aliquot of the suspension (e.g., 100 µl) with an equal volume of 2 × SDS sample buffer.

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 65 KDa proteins.

Figure of protein expression from pETUK

A gene of 65 KDa protein was cloned into pETUK (pETUK/65K). BL21(DE3) cell was transformed with the pETUK/65K, 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 pETUK but not pETUK/65K  
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

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