

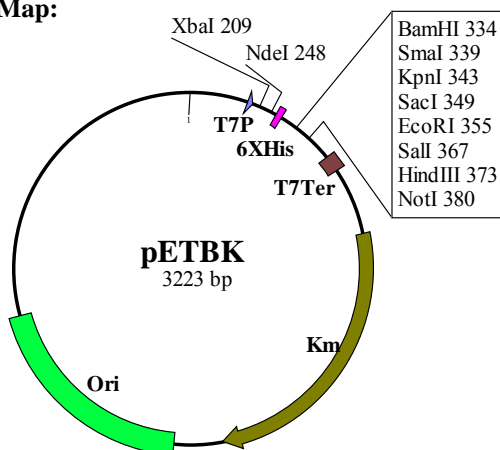
PRODUCT INFORMATION

GENERAL INFORMATION

Product Name : Expression Vector pETBK
Code No. : DV230
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 pETBK with 15 µl of sterile water to make 1 µg/µl plasmid in 1 × TE buffer. After reconstitution, store at - 20 °C

Product Description : pETBK is a medium 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 pETBK vector, and it induces a high-level protein expression from T7 promoter of pETBK. BioDynamics Laboratory Inc. offers several kinds of T7 bacterial expression vectors. Among them, pETBK is standard kanamycin-resistant 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 :	1660-2279
Km :	703-1518

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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pETBK Sequence

CAGACGTTTT	GCAGCAGCAG	TCGCTTCACG	TTCGCTCGCG	TATCGGTGAT	TCATTCTGCT	60
AACCAGTAAG	GCAACCCCGC	CAGCCTAGCC	GGTCCCTCAA	CGACAGGAGC	ACGATCATGC	120
GCACCCGTGG	CCAGGACCCA	ACGCTGCCCC	AGATCTCGAT	CCCGCGAAAT	TAATACGACT	180
CACTATAGGG	AGACCACAAC	GGTTTCCCTC	TAGAAATAAT	TTTGTTTAAC	TTTAAGAAGG	240
AGATATACAT	ATGCGGGGTT	CTCATCATCA	TCATCATCAT	GGTATGGCTA	GCATGACTGG	300
TGGACAGCAA	ATGGGTCTGG	ACGATGACGA	TAAGGATCCC	CGGGTACCGA	GCTCGAATTC	360
GATTTTCGTC	ACAAGCTTAG	CGGCCGCCGT	TTAATCCGGC	TGCTAACAAA	GCCCCGAAAG	420
AAGCTGAGTT	GGCTGCTGCC	ACCGCTGAGC	AATAACTAGC	ATAACCCCTT	GGGGCTCTTA	480
AACGGGTCTT	GAGGGGTTTT	TTGCTGAAAG	GAGGAACTAT	ATCCGGATAA	TGTCATGATA	540
ATAATGGTTT	CTTAGACGTC	AGGTGGCACT	TTTCGGGGAA	ATGTGCGCGG	AACCCCTATT	600
TGTTTATTTT	TCTAAATACA	TTCAAATATG	TATCCGCTCA	TGAGACAATA	ACCCTGATAA	660
ATGCTTATGT	CTGCTTACAT	AAACAGTAAT	ACAAGGGGTG	TTATGAGCCA	TATTCAACGG	720
GAAACGTCTT	GCTCAAGGCC	GCGATTAAAT	TCCAACATGG	ATGCTGATTT	ATATGGGTAT	780
AAATGGGCTC	GCGATAATGT	CGGGCAATCA	GGTGCACAAA	TCTATCGATT	GTATGGGAAG	840
CCCGATGCGC	CAGAGTTGTT	TCTGAAACAT	GGCAAAGGTA	GCGTTGCCAA	TGATGTTACA	900
GATGAGATGG	TCAGACTAAA	CTGGCTGACG	GAATTTATGC	CTCTTCCGAC	CATCAAGCAT	960
TTTATCCGTA	CTCCTGATGA	TGCATGGTTA	CTCACCCTG	CGATCCCAGG	GAAAACAGCA	1020
TTCCAGGTAT	TAGAAGAATA	TCCTGATTCA	GGTGAAAATA	TTGTTGATGC	GCTGGCAGTG	1080
TTCTGCGCC	GGTTGCATTC	GATTCCTGTT	TGTAATTGTC	CTTTTAACAG	CGATCGCGTA	1140
TTTCGTCTCG	CTCAGGCGCA	ATCACGAATG	AATAACGGTT	TGGTTGATGC	GAGTGATTTT	1200
GATGACGAGC	GTAATGGCTG	GCCTGTTGAA	CAAGTCTGGA	AAGAAATGCA	TAAGCTATTG	1260
CCATTCTCAC	CGGATTCAGT	CGTCACTCAT	GGTGATTTCT	CACTTGATAA	CCTTATTTTT	1320
GACGAGGGGA	AATTAATAGG	TTGTATTGAT	GTTGGACGAG	TCGGAATCGC	AGACCGATAC	1380
CAGGATCTTG	CCATCCTATG	GAAC TGCCCT	GGTGAGTTTT	CTCCTTCATT	ACAGAAACGG	1440
CTTTTTCAAA	AATATGGTAT	TGATAATCCT	GATATGAATA	AATTGCAGTT	TCATTTGATG	1500
CTCGATGAGT	TTTTCTAATT	AAAACATATA	TACTTTAGAT	TGATTTAAAA	CTTCATTTTT	1560
AATTTAAAAG	GATCTAGGTG	AAGATCCTTT	TTGATAATCT	CATGACCAA	ATCCCTTAAC	1620
GTGAGTTTTC	GTTCCACTGA	GCGTCAGACC	CCGTAGAAAA	GATCAAAGGA	TCTTCTTGAG	1680
ATCCTTTTTT	TCTGCGCGTA	ATCTGCTGCT	TGCAAACAAA	AAAACCACCG	CTACCAGCGG	1740
TGGTTTTGTT	GCCGGATCAA	GAGCTACCAA	CTCTTTTTTCC	GAAGGTAAct	GGCTTCAGCA	1800
GAGCGCAGAT	ACCAAATACT	GTCCTTCTAG	TGTAGCCGTA	GTTAGGCCAC	CACTTCAAGA	1860
ACTCTGTAGC	ACCGCCTACA	TACCTCGCTC	TGCTAATCCT	GTTACCAGTG	GCTGCTGCCA	1920
GTGGCGATAA	GTCGTGTCTT	ACCGGGTTGG	ACTCAAGACG	ATAGTTACCG	GATAAGGCGC	1980
AGCGGTCTGG	CTGAACGGGG	GGTTCGTGCA	CACAGCCCAG	CTTGGAGCGA	ACGACCTACA	2040
CCGAActGAG	ATACCTACAG	CGTGAGCTAT	GAGAAAGCGC	CACGCTTCCC	GAAGGGAGAA	2100
AGGCGGACAG	GTATCCGGTA	AGCGGCAGGG	TCGGAACAGG	AGAGCGCACG	AGGGAGCTTC	2160
CAGGGGGAAA	CGCCTGGTAT	CTTTATAGTC	CTGTCCGGTT	TCGCCACCTC	TGACTTGAGC	2220
GTCGATTTTT	GTGATGCTCG	TCAGGGGGGC	GGAGCCTATG	GAAAAACGCC	AGCAACGCGG	2280

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CCTTTTTACG	GTCCTGGCC	TTTTGCTGGC	CTTTTGCTCA	CATGTTCTTT	CCTGCGTTAT	2340
CCCCTGATTC	TGTGGATAAC	CGTATTACCG	CCTTTGAGTG	AGCTGATAACC	GCTCGCCGCA	2400
GCCGAACGAC	CGAGCGCAGC	GAGTCAGTGA	GCGAGGAAGC	GGAAGAGCGC	CTGATGCGGT	2460
ATTTTCTCCT	TACGCATCTG	TGCGGTATTT	CACACCGCAT	ATATGGTGCA	CTCTCAGTAC	2520
AATCTGCTCT	GATGCCGCAT	AGTTAAGCCA	GTATACACTC	CGCTATCGCT	ACGTGACTGG	2580
GTCATGGCTG	CGCCCCGACA	CCCGCCAACA	CCCGCTGACG	CGCCCTGACG	GGCTTGTCTG	2640
CTCCCGGCAT	CCGCTTACAG	ACAAGCTGTG	ACCGTCTCCG	GGAGCTGCAT	GTGTCAGAGG	2700
TTTTCACCGT	CATCACCGAA	ACGCGCGAGG	CAGCTGCGGT	AAAGCTCATC	AGCGTGGTCG	2760
TGAAGCGATT	CACAGATGTC	TGCCTGTTCA	TCCGCGTCCA	GCTCGTTGAG	TTTCTCCAGA	2820
AGCGTTAATG	TCTGGCTTCT	GATAAAGCGG	GCCATGTTAA	GGGCGTTTTT	TTCCTGTTTG	2880
GTCACTGATG	CCTCCGTGTA	AGGGGGATTT	CTGTTTATGG	GGGTAATGAT	ACCGATGAAA	2940
CGAGAGAGGA	TGCTCACGAT	ACGGGTACT	GATGATGAAC	ATGCCCGGTT	ACTGGAACGT	3000
TGTGAGGGTA	AACAACCTGGC	GGTATGGATG	CGGCGGGACC	AGAGAAAAAT	CACTCAGGGT	3060
CAATGCCAGC	GCTTCGTTAA	TACAGATGTA	GGTGTTCAC	AGGGTAGCCA	GCAGCATCCT	3120
GCGATGCAGA	TCCGGAACAT	AATGGTGAG	GGCGCTGACT	TCCGCGTTTC	CAGACTTTAC	3180
GAAACACGGA	AACCGAAGAC	CATTCATGTT	GTTGCTCAGG	TCG		3223

PRODUCT INFORMATION

PRODUCT USAGE

Cloning of a gene to pETBK:

Below is the multiple cloning site of pETBK. To express a recombinant protein correctly, it is necessary to clone the gene of interest in frame with an N-terminal peptide of pETBK. The start codon of pETBK is boxed ATG in the below figure. Digest pETBK 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 pETBK 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. In the transformation, recombinant cells should be selected on LB agar plates containing 15-25 μ g/ml of kanamycin., because higher concentration of kanamycin often retarded cell growth on the agar plates. Recombinant plasmids derived from pETBK 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 217
AspProAlaL ysLeuIleAr gLeuThrIle GlyArgProG lnArgPhePr oSerArgAsn
                NdeI                                6xHis
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 TTCGATTTCC TCGACAAGCT TAGCGGCCGC CGTTTAAATCC 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. 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 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.

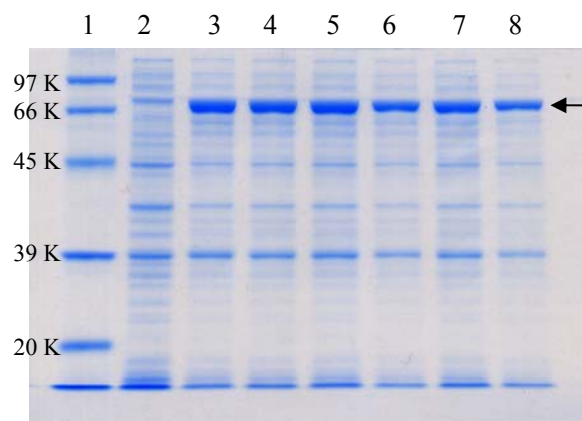
2. Mix an aliquot of the suspension (e.g., 100 µl) with an equal volume of 2 × SDS sample buffer.

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 65 KDa proteins.

Figure of protein expression from pETBK

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

● Purchaser Notification

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