PRODUCT INFORMATION

Product Name : pET Expression pack (pETBK)
Code No. : DS255BK
Kit Component :

Component	Code No.	Contents
pET Expression vector pETBK	DV230	15 µg (lyophilized plasmid contains salt of TE buffer)
Zip Competent Cell BL21(DE3)	DS255	3 tubes (100 µL/tube), transfromation efficiency: 2×10^6 cfu/µg (pUC19)

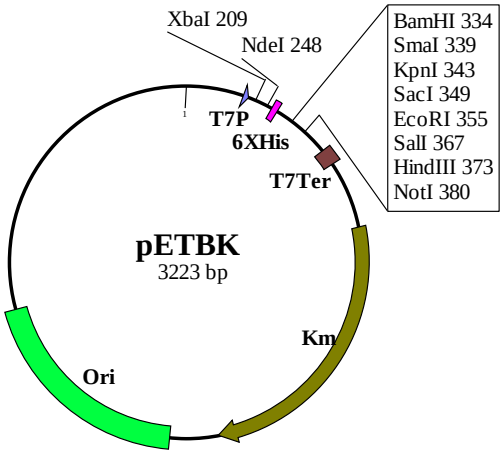
Storage : Store at -80ºC

Product Description:

1) pET Expression vector pETBK

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

| 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 - 20ºC

PRODUCT INFORMATION

| 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

| pETBK 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
ATGCTTATGT CTGCTTACAT AAACAGTAAT ACAAGGGGTG TTATGAGCCA TATTCAACGG   720
GAAACGTCTT GCTCAAGGCC GCGATTAAAT TCCAACATGG ATGCTGATTT ATATGGGTAT   780
AAATGGGCTC GCGATAATGT CGGGCAATCA GGTGCGACAA TCTATCGATT GTATGGGAAG   840
CCCGATGCGC CAGAGTTGTT TCTGAAACAT GGCAAAGGTA GCGTTGCCAA TGATGTTACA   900
GATGAGATGG TCAGACTAAA CTGGCTGACG GAATTTATGC CTCTTCCGAC CATCAAGCAT   960
TTTATCCGTA CTCCTGATGA TGCATGGTTA CTCACCACTG CGATCCCAGG GAAAACAGCA  1020
TTCCAGGTAT TAGAAGAATA TCCTGATTCA GGTGAAAATA TTGTTGATGC GCTGGCAGTG  1080
TTCCTGCGCC 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 GAACTGCCTC GGTGAGTTTT CTCCTTCATT ACAGAAACGG  1440
CTTTTTCAAA AATATGGTAT TGATAATCCT GATATGAATA AATTGCAGTT TCATTTGATG  1500
CTCGATGAGT TTTTCTAATT AAAACATATA TACTTTAGAT TGATTTAAAA CTTCATTTTT  1560
AATTTAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAAA ATCCCTTAAC  1620
GTGAGTTTTC GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG  1680
ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCACCG CTACCAGCGG  1740
TGGTTTGTTT GCCGGATCAA GAGCTACCAA CTCTTTTTCC GAAGGTAACT GGCTTCAGCA  1800
GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA  1860
ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA  1920
GTGGCGATAA GTCGTGTCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC  1980
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AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCCAG CTTGGAGCGA ACGACCTACA  2040
CCGAACTGAG ATACCTACAG CGTGAGCTAT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA  2100
AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG AGAGCGCACG AGGGAGCTTC  2160
CAGGGGGAAA CGCCTGGTAT CTTTATAGTC CTGTCGGGTT TCGCCACCTC TGACTTGAGC  2220
GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG  2280
CCTTTTTACG GTTCCTGGCC TTTTGCTGGC CTTTTGCTCA CATGTTCTTT CCTGCGTTAT  2340
CCCCTGATTC TGTGGATAAC CGTATTACCG CCTTTGAGTG AGCTGATACC 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 GGGCGGTTTT TTCCTGTTTG  2880
GTCACTGATG CCTCCGTGTA AGGGGGATTT CTGTTCATGG GGGTAATGAT ACCGATGAAA  2940
CGAGAGAGGA TGCTCACGAT ACGGGTTACT GATGATGAAC ATGCCCGGTT ACTGGAACGT  3000
TGTGAGGGTA AACAACTGGC GGTATGGATG CGGCGGGACC AGAGAAAAAT CACTCAGGGT  3060
CAATGCCAGC GCTTCGTTAA TACAGATGTA GGTGTTCCAC AGGGTAGCCA GCAGCATCCT  3120
GCGATGCAGA TCCGGAACAT AATGGTGCAG GGCGCTGACT TCCGCGTTTC CAGACTTTAC  3180
GAAACACGGA AACCGAAGAC CATTCATGTT GTTGCTCAGG TCG                    3223
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PRODUCT INFORMATION

2) Zip Competent Cell BL21(DE3)

The Zip Competent Cell BL21(DE3) is designed for a rapid transformation procedure. It requires neither heat shock nor culture after heat shock. Transformation of the Zip Competent Cell BL21(DE3) is completed within about 5 minutes. The time-saving procedure is a great benefit for researchers and experimenters.

The strain, BL21(DE3), is one of the popular strains for the protein expression. BL21(DE3) contains the λDE3 lysogen, which expresses T7 RNA polymerase under the control of the *lac*UV5 promoter[1)]. Upon addition of isopropyl-1-thio-β-D-galactopyranoside (IPTG), T7 RNA polymerase is expressed and it induces a high-level protein expression from T7 promoter driven expression vectors. *E. coli* BL21(DE3) strain is a derivative of *E. coli* B strain and lacks both the *lon* protease and the *ompT* membrane protease which may degrade expressed proteins.

| Genotype of *E. coli* strain BL21(DE3) :

F^- *ompT hsdS*(r_B^- m_B^-) *gal dcm* λ(DE3)　(λ(DE3): *lac*I, *lac*UV5-T7 gene 1, *ind*1, *sam*7, *nin*5)

| Storage condition of Zip Competent Cell BL21(DE3) :

Stable at -80℃ with little or no loss in transformation efficiency for 12 months from the date of receipt. Competent cells are sensitive to variation in temperature. Must be stored at -80°C. Upon receipt, store the Zip Competent Cell BL21(DE3) in a freezer at -80°C directly from the dry ice shipping box.

| Handling of competent cells :

• Competent cells are sensitive to mechanical shock. Excessive mixing should be avoided.

• After thawing competent cells on ice, cells tend to lose transformation efficiency gradually. Transformation should be started immediately after the cells have thawed on ice.

• Use of refrozen competent cells is not recommended.

| Quality Control of Zip Competent Cell BL21(DE3):

Transformation was carried out according to the method described in this Product Information using supercoiled pUC19 plasmid. Transformants were plated on LB plates containing 50 μg/mL ampicillin. The efficiency was confirmed to be greater than 2×10^6 cfu/μg.

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 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 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.

```
              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
```

EK: Enterokinase recognition sequence (AspAspAspAspLys↓)
ATG: start codon TAA : stop codon

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http://www.biodynamics.co.jp

PRODUCT INFORMATION

| Transformation:

● Materials to be supplied by user

- LB plates containing an appropriate antibiotic
- Ice bucket with ice
- Sterile spreaders

● Transformation procedure

1. Thaw one tube of competent cells on ice. One tube contains 100 µL of cells for each transformation.
2. Add DNA sample* directly into the competent cells and mix by flicking the tube.

*1 The volume of DNA sample should not exceed 5 % of that of competent cells (i.e. for 100 µL of competent cells, use ≦5 µL).

*2 If it is predicted that you will have too many colonies on the LB plate, dilute the plasmid solution with autoclaved water.

3. Leave the tube on ice for 5 minutes.
4. Spread the 100 µl of the cells to a 37ºC pre-warmed LB agar plate containing an appropriate antibiotic.
5. Incubate the plates at 37°C overnight.

| 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.

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.

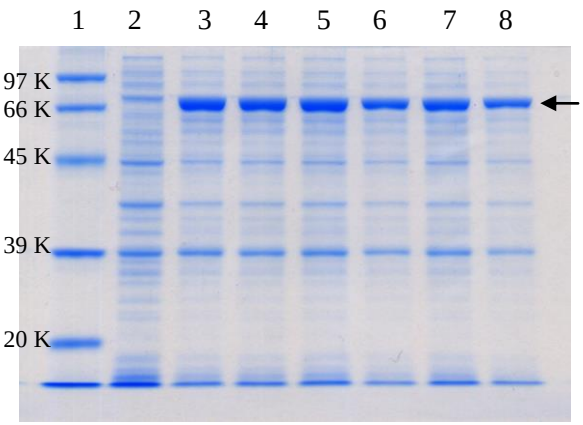
4. After the induction, harvest the cells. To analyze the expression, before harvesting the cells, transfer an aliquot of the culture (e.g., 1 mL) and centrifuge it to precipitate the cells.

| 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

PRODUCT INFORMATION



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:
   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.

Reference:

1) Studier, F.W. and Moffatt, B.A., *J. Mol. Biol.* 189 (1986) 113–130.
2) Moffatt, B.A. and Studier, F.W., Cell 49 (1987) 221-227
3) Pan, S. and Malcom, B.A., *BioTechniques* 29 (2000), 1234–1238

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.

PRODUCT INFORMATION

Related Products:

DS250	Competent Cell BL21(DE3)	DS258	Electrocompetent Cell BL21(DE3)
DS260	Competent Cell BL21(DE3)pLysS		

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 Stated 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 maybe 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.