PRODUCT INFORMATION

Product Name : pET Expression pack (pETUK)
Code No. : DS255UK
Kit Component :

Component	Code No.	Contents
pET Expression vector pETUK	DV220	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)

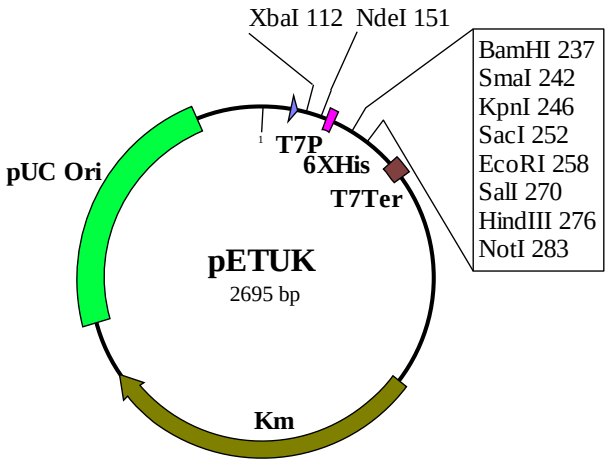
Storage : Store at -80ºC

Product Description:

1) pET Expression vector pETUK

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

| 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 - 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

| pETUK Sequence

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GCGCCCAATA CGCAAACCGC CTCTCCCCGC GCGTTGGCCG ATTCATTAAT GCAGGATCTC    60
GATCCCGCGA AATTAATACG ACTCACTATA GGGAGACCAC AACGGTTTCC CTCTAGAAAT   120
AATTTTGTTT AACTTTAAGA AGGAGATATA CATATGCGGG GTTCTCATCA TCATCATCAT   180
CATGGTATGG CTAGCATGAC TGGTGGACAG CAAATGGGTC GGGACGATGA CGATAAGGAT   240
CCCCGGGTAC CGAGCTCGAA TTCGATTTCG TCGACAAGCT TAGCGGCCGC CGTTTAATCC   300
GGCTGCTAAC AAAGCCCGAA AGGAAGCTGA GTTGGCTGCT GCCACCGCTG AGCAATAACT   360
AGCATAACCC CTTGGGGCCT CTAAACGGGT CTTGAGGGGT TTTTTGCTGA AAGGAGGAAC   420
TATATCCGGA TCTGGCGTAA TAGCGAAGAG GCCCGCACCG ATCGCCCTTC CCAACAGTTG   480
CGCAGCCTGA ATGGCGAATG GCGCCTGATG CGGTATTTTC TCCTTACGCA TCTGTGCGGT   540
ATTTCACACC GCATCTGGTG CACTCTCAGT ACAATCTGCT CTGATGCCGC ATAGTTAAGC   600
CAGCCCCGAC ACCCGCCAAC ACCCGCTGAC GCGCCCTGAC GGGCTTGTCT GCTCCCGGCA   660
TCCGCTTACA GACAAGCTGT GACCGTCTCC GGGAGCTGCA TGTGTCAGAG GTTTTCACCG   720
TCATCACCGA AACGCGCGAG ACGAAAGGGC CTCGTGATAC GCCTATTTTT ATAGGTTAAT   780
GTCATGATAA TAATGGTTTC TTAGACGTCA GGTGGCACTT TTCGGGGAAA TGTGCGCGGA   840
ACCCCTATTT GTTTATTTTT CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA   900
CCCTGATAAA TGCTTATGTC TGCTTACATA AACAGTAATA CAAGGGGTGT TATGAGCCAT   960
ATTCAACGGG AAACGTCTTG 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 TCACCACTGC GATCCCAGGG  1260
AAAACAGCAT TCCAGGTATT AGAAGAATAT CCTGATTCAG GTGAAAATAT TGTTGATGCG  1320
CTGGCAGTGT TCCTGCGCCG GTTGCATTCG ATTCCTGTTT GTAATTGTCC TTTTAACAGC  1380
GATCGCGTAT TTCGTCTCGC TCAGGCGCAA TCACGAATGA ATAACGGTTT GGTTGATGCG  1440
AGTGATTTTG ATGACGAGCG TAATGGCTGG CCTGTTGAAC AAGTCTGGAA AGAAATGCAT  1500
AAGCTATTGC CATTCTCACC GGATTCAGTC GTCACTCATG GTGATTTCTC ACTTGATAAC  1560
CTTATTTTTG ACGAGGGGAA ATTAATAGGT TGTATTGATG TTGGACGAGT CGGAATCGCA  1620
GACCGATACC AGGATCTTGC CATCCTATGG AACTGCCTCG GTGAGTTTTC TCCTTCATTA  1680
CAGAAACGGC TTTTTCAAAA ATATGGTATT GATAATCCTG ATATGAATAA ATTGCAGTTT  1740
CATTTGATGC TCGATGAGTT TTTCTAATTA AAACATATAT ACTTTAGATT GATTTAAAAC  1800
TTCATTTTTA ATTTAAAAGG ATCTAGGTGA AGATCCTTTT TGATAATCTC ATGACCAAAA  1860
TCCCTTAACG TGAGTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAGGAT  1920
CTTCTTGAGA TCCTTTTTTT CTGCGCGTAA TCTGCTGCTT GCAAACAAAA AAACCACCGC  1980
TACCAGCGGT GGTTTGTTTG CCGGATCAAG AGCTACCAAC TCTTTTTCCG AAGGTAACTG  2040
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PRODUCT INFORMATION

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GCTTCAGCAG AGCGCAGATA CCAAATACTG TTCTTCTAGT GTAGCCGTAG TTAGGCCACC  2100
ACTTCAAGAA CTCTGTAGCA CCGCCTACAT ACCTCGCTCT GCTAATCCTG TTACCAGTGG  2160
CTGCTGCCAG TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG  2220
ATAAGGCGCA GCGGTCGGGC TGAACGGGGG GTTCGTGCAC 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 TTCCTGGCCT TTTGCTGGCC TTTTGCTCAC ATGTTCTTTC  2580
CTGCGTTATC CCCTGATTCT GTGGATAACC GTATTACCGC CTTTGAGTGA GCTGATACCG  2640
CTCGCCGCAG CCGAACGACC GAGCGCAGCG AGTCAGTGAG CGAGGAAGCG GAAGA       2695
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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 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 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 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.

```
              T7 promoter                                  XbaI
GATCCCGCGA AATTAATACG ACTCACTATA GGGAGACCAC AACGGTTTCC CTCTAGAAAT   120
AspProAlaL ysLeuIleAr gLeuThrIle GlyArgProG lnArgPhePr oSerArgAsn
                                  NdeI              6×His
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 TTCGATTTCG TCGACAAGCT TAGCGGCCGC CGTTTAATCC   300
  SmaI        SacI                    HindIII
ProArgValP roSerSerAs nSerIleSer SerThrSerL euAlaAlaAl aVal***Ser
```

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

9-7 HONGO 2-CHOME,BUNKYO-KU,TOKYO113-0033,JAPAN
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.

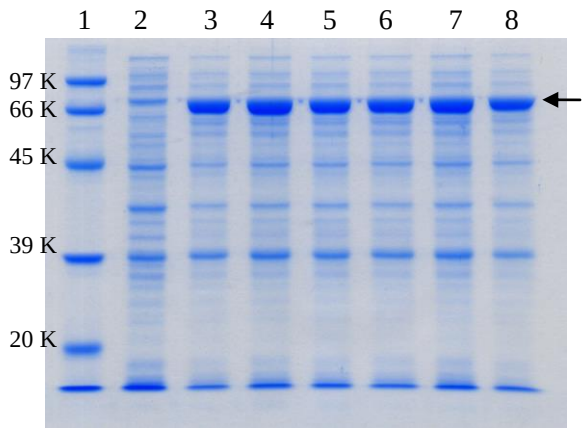
4. After the induction, harvest the cells. To analyze the expression, before harvesting the cells, transfer an aliquot of the culture (e.g., 1 mL) and centrifuge it to precipitate the cells.

| 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 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:
   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:

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