PRODUCT INFORMATION

Product Name : pET Expression pack (pETUA)
Code No. : DS255UA
Kit Component :

Component	Code No.	Contents
pET Expression vector pETUA	DV200	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)

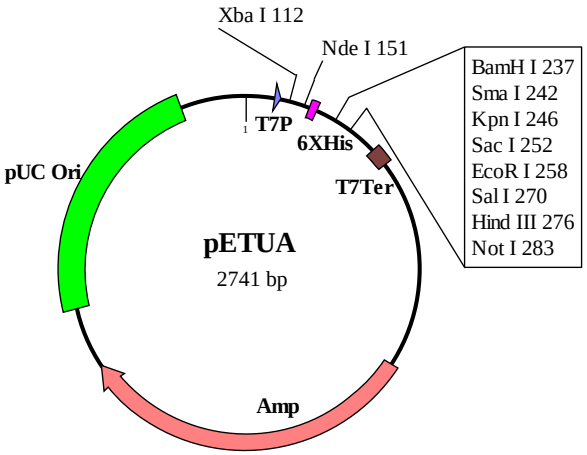
Storage : Store at -80ºC

Product Description:

1) pET Expression vector pETUA

pETUA is a high copy number, ampicillin 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 pETUA vector, and it induces a high-level protein expression from T7 promoter of pETUA. BioDynamics Laboratory Inc. offers several kinds of T7 bacterial expression vectors. Among them, pETUA is suitable for a high level expression of non-toxic proteins and the high copy number of pETUA 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 :	1955-2574
Amp (bla) :	940-1800

| Reconstitution : Resuspend the lyophilized pETUA 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

| pETUA 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 TGCTTCAATA ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT   960
GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCCTTCCTG TTTTTGCTCA CCCAGAAACG  1020
CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG  1080
GATCTCAACA GCGGTAAGAT CCTTGAGAGT TTTCGCCCCG AAGAACGTTT TCCAATGATG  1140
AGCACTTTTA AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTATTGACGC CGGGCAAGAG  1200
CAACTCGGTC GCCGCATACA CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA  1260
GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG  1320
AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC  1380
GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG ATCGTTGGGA ACCGGAGCTG  1440
AATGAAGCCA TACCAAACGA CGAGCGTGAC ACCACGATGC CTGTAGCAAT GGCAACAACG  1500
TTGCGCAAAC TATTAACTGG CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC  1560
TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG  1620
TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG  1680
GGGCCAGATG GTAAGCCCTC CCGTATCGTA GTTATCTACA CGACGGGGAG TCAGGCAACT  1740
ATGGATGAAC GAAATAGACA GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA  1800
CTGTCAGACC AAGTTTACTC ATATATACTT TAGATTGATT TAAAACTTCA TTTTTAATTT  1860
AAAAGGATCT AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG  1920
TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT  1980
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PRODUCT INFORMATION

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TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT  2040
TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG  2100
CAGATACCAA ATACTGTTCT TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT  2160
GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC  2220
GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG  2280
TCGGGCTGAA CGGGGGGTTC GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA  2340
CTGAGATACC TACAGCGTGA GCTATGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG  2400
GACAGGTATC CGGTAAGCGG CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG  2460
GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA  2520
TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT  2580
TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT TCTTTCCTGC GTTATCCCCT  2640
GATTCTGTGG ATAACCGTAT TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA  2700
ACGACCGAGC GCAGCGAGTC AGTGAGCGAG GAAGCGGAAG A                      2741
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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 pETUA:

Below is the multiple cloning site of pETUA. To express a recombinant protein correctly, it is necessary to clone the gene of interest in frame with an N-terminal peptide of pETUA. The start codon of pETUA is boxed ATG in the below figure. Digest pETUA 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 pETUA 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. Recombinant plasmids derived from pETUA 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              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.

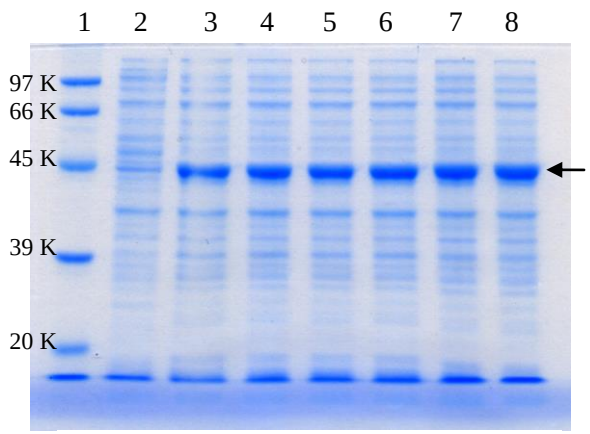
4. After the induction, harvest the cells. To analyze the expression, before harvesting the cells, transfer an aliquot of the culture (e.g., 1 mL) and centrifuge it to precipitate the cells.

| 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 44 KDa proteins.

Figure of protein expression from pETUA

A gene of 44 KDa protein was cloned into pETUA (pETUA/44K). BL21(DE3) cell was transformed with the pETUA/44K, 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 pETUA but not pETUA/44K
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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