PRODUCT INFORMATION

Product Name : pET Expression pack (pETIA)
Code No. : DS255IA
Kit Component :

Component	Code No.	Contents
pET Expression vector pETIA	DV215	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)

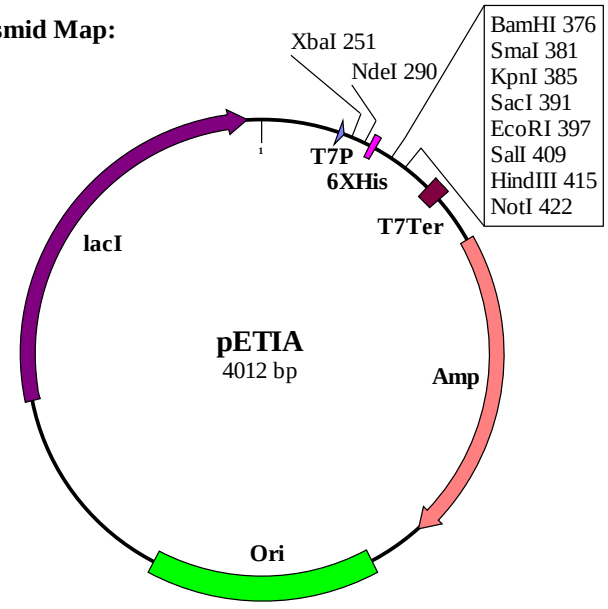
Storage : Store at -80ºC

Product Description:

1) pET Expression vector pETIA

pETIA is a medium copy number, ampicillin resistant, stringent controllable 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* cell. 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 pETIA vector, and it induces a high-level protein expression from T7 promoter of pETIA. The pETIA has a lacI gene, which represses T7 RNA polymerase gene in the absence of IPTG. The regulation with lac repressor is beneficial to repress a basal level protein expression and to maintain a recombinant plasmid in BL21 (DE3) cell.

| Plasmid Map:



T7 promoter :	213-229
T7 transcription start :	230
His Tag :	305-322
T7 terminator :	498-545
lacI :	2891-3973
pMB1 ori :	1695-2314
Amp (bla) :	680-1540

| Reconstitution : Resuspend the lyophilized pETIA 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

| pETIA Sequence

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GTTTGACAGC TTATCATCGA CTGCACGGTG CACCAATGCT TCTGGCGTCA GGCAGCCATC    60
GGAAGCTGTG GTATGGCTGT GCAGGTCGTA AATCACTGCA TAATTCGTGT CGCTCAAGGC   120
GCACTCCCGT TCTGGATAAT GTTTTTTGCG CCGACATCAT AACGGTTCTG GCAAATATTC   180
TGAAATGAGC TGAGATCTCG ATCCCGCGAA ATTAATACGA CTCACTATAG GGAGACCACA   240
ACGGTTTCCC TCTAGAAATA ATTTTGTTTA ACTTTAAGAA GGAGATATAC ATATGCGGGG   300
TTCTCATCAT CATCATCATC ATGGTATGGC TAGCATGACT GGTGGACAGC AAATGGGTCG   360
GGACGATGAC GATAAGGATC CCCGGGTACC GAGCTCGAAT TCGATTTCGT CGACAAGCTT   420
AGCGGCCGCC GTTTAATCCG GCTGCTAACA AAGCCCGAAA GGAAGCTGAG TTGGCTGCTG   480
CCACCGCTGA GCAATAACTA GCATAACCCC TTGGGGCCTC TAAACGGGTC TTGAGGGGTT   540
TTTTGCTGAA AGGAGGAACT ATATCCGGAT GCGTTTCTAC AAACTCTTTT GTTTATTTTT   600
CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA   660
ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT GTCGCCCTTA TTCCCTTTTT   720
TGCGGCATTT TGCCTTCCTG TTTTTGCTCA CCCAGAAACG CTGGTGAAAG TAAAAGATGC   780
TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT   840
CCTTGAGAGT TTTCGCCCCG AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT   900
ATGTGGCGCG GTATTATCCC GTGTTGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA   960
CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG  1020
CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG AGTGATAACA CTGCGGCCAA  1080
CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG  1140
GGATCATGTA ACTCGCCTTG ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA  1200
CGAGCGTGAC ACCACGATGC CTGTAGCAAT GGCAACAACG TTGCGCAAAC TATTAACTGG  1260
CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC TGGATGGAGG CGGATAAAGT  1320
TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG TTTATTGCTG ATAAATCTGG  1380
AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC  1440
CCGTATCGTA GTTATCTACA CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA  1500
GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA CTGTCAGACC AAGTTTACTC  1560
ATATATACTT TAGATTGATT TAAAACTTCA TTTTTAATTT AAAAGGATCT AGGTGAAGAT  1620
CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG TTTTCGTTCC ACTGAGCGTC  1680
AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC GCGTAATCTG  1740
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CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT TGTTTGCCGG ATCAAGAGCT  1800
ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT  1860
TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC CTACATACCT  1920
CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC GATAAGTCGT GTCTTACCGG  1980
GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTC  2040
GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA  2100
GCTATGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG  2160
CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA  2220
TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA TTTTTGTGAT GCTCGTCAGG  2280
GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG  2340
CTGGCCTTTT GCTCACATGT TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT  2400
TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC  2460
AGTGAGCGAG GAAGCGGAAG AGCGCCTGAT GCGGTATTTT CTCCTTACGC ATCTGTGCGG  2520
TATTTCACAC CGCATATATG GTGCACTCTC AGTACAATCT GCTCTGATGC CGCATAGTTA  2580
AGCCAGTATA CACTCCGCTA TCGCTACGTG ACTGGGTCAT GGCTGCGCCC CGACACCCGC  2640
CAACACCCGC TGACGCGCCC TGACGGGCTT GTCTGCTCCC GGCATCCGCT TACAGACAAG  2700
CTGTGACCGT CTCCGGGAGC TGCATGTGTC AGAGGTTTTC ACCGTCATCA CCGAAACGCG  2760
CGAGGCAGCA GATCAATTCG CGCGCGAAGG CGAAGCGGCA TGCATTTACG TTGACACCAT  2820
CGAATGGTGC AAAACCTTTC GCGGTATGGC ATGATAGCGC CCGGAAGAGA GTCAATTCAG  2880
GGTGGTGAAT GTGAAACCAG TAACGTTATA CGATGTCGCA GAGTATGCCG GTGTCTCTTA  2940
TCAGACCGTT TCCCGCGTGG TGAACCAGGC CAGCCACGTT TCTGCGAAAA CGCGGGAAAA  3000
AGTGGAAGCG GCGATGGCGG AGCTGAATTA CATTCCCAAC CGCGTGGCAC AACAACTGGC  3060
GGGCAAACAG TCGTTGCTGA TTGGCGTTGC CACCTCCAGT CTGGCCCTGC ACGCGCCGTC  3120
GCAAATTGTC GCGGCGATTA AATCTCGCGC CGATCAACTG GGTGCCAGCG TGGTGGTGTC  3180
GATGGTAGAA CGAAGCGGCG TCGAAGCCTG TAAAGCGGCG GTGCACAATC TTCTCGCGCA  3240
ACGCGTCAGT GGGCTGATCA TTAACTATCC GCTGGATGAC CAGGATGCCA TTGCTGTGGA  3300
AGCTGCCTGC ACTAATGTTC CGGCGTTATT TCTTGATGTC TCTGACCAGA CACCCATCAA  3360
CAGTATTATT TTCTCCCATG AAGACGGTAC GCGACTGGGC GTGGAGCATC TGGTCGCATT  3420
GGGTCACCAG CAAATCGCGC TGTTAGCGGG CCCATTAAGT TCTGTCTCGG CGCGTCTGCG  3480
TCTGGCTGGC TGGCATAAAT ATCTCACTCG CAATCAAATT CAGCCGATAG CGGAACGGGA  3540
AGGCGACTGG AGTGCCATGT CCGGTTTTCA ACAAACCATG CAAATGCTGA ATGAGGGCAT  3600
CGTTCCCACT GCGATGCTGG TTGCCAACGA TCAGATGGCG CTGGGCGCAA TGCGCGCCAT  3660
TACCGAGTCC GGGCTGCGCG TTGGTGCGGA TATCTCGGTA GTGGGATACG ACGATACCGA  3720
AGACAGCTCA TGTTATATCC CGCCGTTAAC CACCATCAAA CAGGATTTTC GCCTGCTGGG  3780
GCAAACCAGC GTGGACCGCT TGCTGCAACT CTCTCAGGGC CAGGCGGTGA AGGGCAATCA  3840
GCTGTTGCCC GTCTCACTGG TGAAAAGAAA AACCACCCTG GCGCCCAATA CGCAAACCGC  3900
CTCTCCCCGC GCGTTGGCCG ATTCATTAAT GCAGCTGGCA CGACAGGTTT CCCGACTGGA  3960
AAGCGGGCAG TGAGCGCAAC GCAATTAATG TAAGTTAGCG CGAATTGATC TG          4012
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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 -80ºC. Upon receipt, store the Zip Competent Cell BL21(DE3) in a freezer at -80ºC 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 pETIA:

Below is the multiple cloning site of pETIA. To express a recombinant protein correctly, it is necessary to clone the gene of interest in frame with an N-terminal peptide of pETIA. The start codon of pETIA is boxed ATG in the below figure. Digest pETIA 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 pETIA 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 pETIA 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   259
AspProAlaL ysLeuIleAr gLeuThrIle GlyArgProG lnArgPhePr oSerArgAsn
                                  NdeI              6×His
AATTTTGTTT AACTTTAAGA AGGAGATATA CATATGCGGG GTTCTCATCA TCATCATCAT   319
AsnPheVal* **Leu***Gl uGlyAspIle HisMetArgG lySerHisHi sHisHisHis
                                                    EK      BamHI
CATGGTATGG CTAGCATGAC TGGTGGACAG CAAATGGGTC GGGACGATGA CGATAAGGAT   379
HisGlyMetA laSerMetTh rGlyGlyGln GlnMetGlyA rgAspAspAs pAspLysAsp
     KpnI          EcoRI        SalI            NotI            ↑
CCCCGGGTAC CGAGCTCGAA TTCGATTTCG TCGACAAGCT TAGCGGCCGC CGTTTAATCC   439
  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

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.

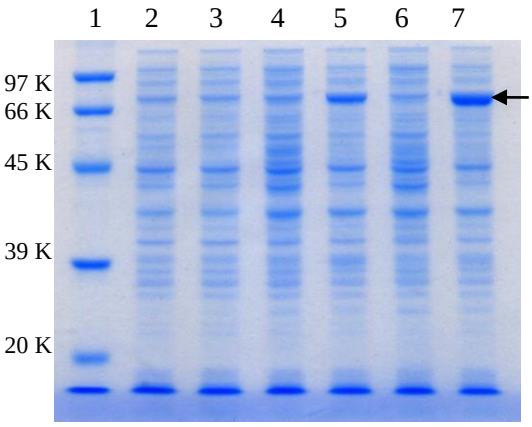
4. After the induction, harvest the cells. To analyze the expression, before harvesting the cells, transfer an aliquot of the culture (e.g., 1 mL) and centrifuge it to precipitate the cells.

| 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 70 KDa proteins. Only inducted cells expressed 70 KDa proteins.

Figure of protein expression from pETIA

A gene of 70 KDa protein was cloned into pETIA (pETIA/70K). BL21(DE3) cell was transformed with the pETIA/70K, one of colonies were cultured overnight and transferred to two tubes (#1, #2) containing culture medium. IPTG was added to only tube #2 when the OD_{600} reaches 0.5. At each stage, OD_{600} of the culture was determined and the same amount of cells were lysed and subjected to 10 % polyacrylamide gel SDS electrophoresis.

Lane 1: DynaMarker Protein Eco (#DM610)
Lane 2, 3 : Cells from tubes #1 and 2 before induction.
Lane 4 : Cells (tubes #1), two hours after OD0.5.
Lane 5 :Cells (tubes #2), two hours after induction
Lane 6 : Cells (tubes #1), 4 hours after OD0.5.
Lane 7 :Cells (tubes #2), 4 hours after induction

● 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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