

PRODUCT INFORMATION

Product Name : pET Expression pack (pETBA)

Code No. : DS255BA

Kit Component :

Component	Code No.	Contents
pET Expression vector pETBA	DV210	15 µg (lyophilized plasmid contains salt of TE buffer)
Zip Competent Cell BL21(DE3)	DS255	3 tubes (100 µL/tube), transformation efficiency: 2×10^6 cfu/µg (pUC19)

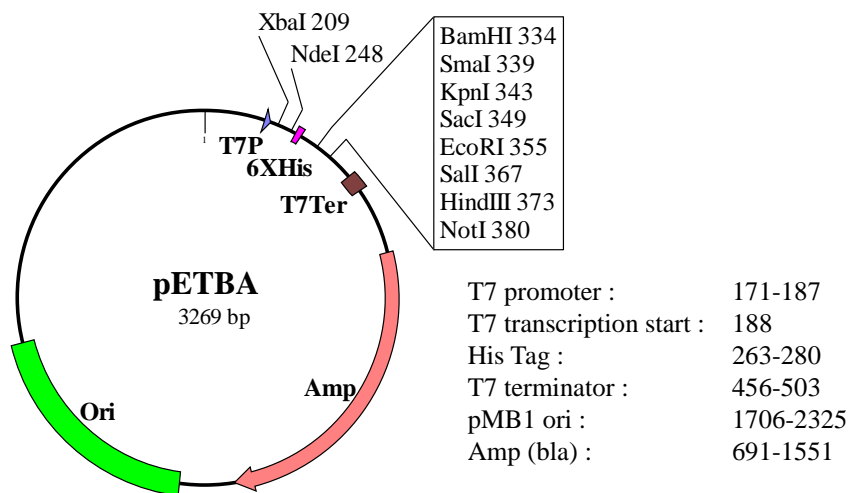
Storage : Store at -80°C

Product Description:

1) pET Expression vector pETBA

pETBA is a medium 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* 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 pETBA vector, and it induces a high-level protein expression from T7 promoter of pETBA. BioDynamics Laboratory Inc. offers several kinds of T7 bacterial expression vectors. Among them, pETBA is standard vector for a high level expression of proteins.

| Plasmid Map:



| Reconstitution : Resuspend the lyophilized pETBA with 15 µl of sterile water to make 1 µg/µl plasmid in 1 × TE buffer. After reconstitution, store at -20°C

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| 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

| pETBA Sequence

CAGACGTTTT	GCAGCAGCAG	TCGCTTCACG	TTCGCTCGCG	TATCGGTGAT	TCATTCTGCT	60
AACCAGTAAG	GCAACCCCGC	CAGCCTAGCC	GGGTCCCTCAA	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	ATGGGTTCGGG	ACGATGACGA	TAAGGATCCC	CGGGTACCGA	GCTCGAATTC	360
GATTTTCGTCG	ACAAGCTTAG	CGGCCGCCGT	TTAATCCGGC	TGCTAACAAA	GCCCCGAAAGG	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
ATGCTTCAAT	AATATTGAAA	AAGGAAGAGT	ATGAGTATTC	AACATTTCCG	TGTCGCCCTT	720
ATTCCCTTTT	TTGCGGCATT	TTGCCTTCCT	GTTTTTGCTC	ACCCAGAAAC	GCTGGTGAAA	780
GTA AAAAGATG	CTGAAGATCA	GTTGGGTGCA	CGAGTGGGTT	ACATCGAACT	GGATCTCAAC	840
AGCGGTAAGA	TCCTTGAGAG	TTTTTCGCCC	GAAGAACGTT	TTCCAATGAT	GAGCACTTTT	900
AAAGTTCTGC	TATGTGGCGC	GGTATTATCC	CGTGTGACG	CCGGGCAAGA	GCAACTCGGT	960
CGCCGCATAC	ACTATTCTCA	GAATGACTTG	GTTGAGTACT	CACCAGTCAC	AGAAAAGCAT	1020
CTTACGGATG	GCATGACAGT	AAGAGAATTA	TGCAGTGCTG	CCATAACCAT	GAGTGATAAC	1080
ACTGCGGCCA	ACTTACTTCT	GACAACGATC	GGAGGACCGA	AGGAGCTAAC	CGCTTTTTTTG	1140
CACAACATGG	GGGATCATGT	AACTCGCCTT	GATCGTTGGG	AACCGGAGCT	GAATGAAGCC	1200
ATACCAAACG	ACGAGCGTGA	CACCACGATG	CCTGCAGCAA	TGGCAACAAC	GTTGCGCAAA	1260
CTATTAAC TG	GCGAACTACT	TACTCTAGCT	TCCC GGCAAC	AATTAATAGA	CTGGATGGAG	1320
GCGGATAAAG	TTGCAGGACC	ACTTCTGCGC	TCGGCCCTTC	CGGCTGGCTG	GTTTATTGCT	1380
GATAAAATCTG	GAGCCGGTGA	GCGTGGGTCT	CGCGGTATCA	TTGCAGCACT	GGGGCCAGAT	1440
GGTAAGCCCT	CCC GTATCGT	AGTTATCTAC	ACGACGGGGA	GTCAGGCAAC	TATGGATGAA	1500
CGAAATAGAC	AGATCGCTGA	GATAGGTGCC	TCACTGATTA	AGCATTGGTA	ACTGTCAGAC	1560
CAAGTTTACT	CATATATACT	TTAGATTGAT	TTAAAAC TTC	ATTTTTAATT	TAAAAGGATC	1620
TAGGTGAAGA	TCCTTTTTGA	TAATCTCATG	ACCAAATCC	CTTAACGTGA	GTTTTCGTTC	1680
CACTGAGCGT	CAGACCCCGT	AGAAAAGATC	AAAGGATCTT	CTTGAGATCC	TTTTTTTCTG	1740
CGCGTAATCT	GCTGCTTGCA	AACAAAAAAA	CCACCGCTAC	CAGCGGTGGT	TTGTTTGCCG	1800
GATCAAGAGC	TACCAACTCT	TTTTCCGAAG	GTAAC TGGCT	TCAGCAGAGC	GCAGATACCA	1860
AATACTGTCC	TTCTAGTGTA	GCCGTAGTTA	GGCCACC ACT	TCAAGAACTC	TGTAGCACCG	1920
CCTACATAACC	TCGCTCTGCT	AATCCTGTTA	CCAGTGGCTG	CTGCCAGTGG	CGATAAGTCG	1980

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TGTCTTACCG	GGTTGACTC	AAGACGATAG	TTACCGGATA	AGGCGCAGCG	GTCGGGCTGA	2040
ACGGGGGGTT	CGTGCACACA	GCCCAGCTTG	GAGCGAACGA	CCTACACCGA	ACTGAGATAC	2100
CTACAGCGTG	AGCTATGAGA	AAGCGCCACG	CTTCCCAGAG	GGAGAAAGGC	GGACAGGTAT	2160
CCGGTAAGCG	GCAGGGTCGG	AACAGGAGAG	CGCACGAGGG	AGCTTCCAGG	GGGAAACGCC	2220
TGGTATCTTT	ATAGTCCTGT	CGGGTTTCGC	CACCTCTGAC	TTGAGCGTCG	ATTTTTGTGA	2280
TGCTCGTCAG	GGGGCGGAG	CCTATGAAAA	AACGCCAGCA	ACGCGGCCTT	TTTACGGTTC	2380
CTGGCCTTTT	GCTGGCCTTT	TGCTCACATG	TTCTTTCCCTG	CGTTATCCCC	TGATTCTGTG	2400
GATAACCGTA	TTACCGCCTT	TGAGTGAGCT	GATACCGCTC	GCCGCAGCCG	AACGACCGAG	2460
CGCAGCGAGT	CAGTGAGCGA	GGAAGCGGAA	GAGCGCCTGA	TGCGGTATTT	TCTCCTTACG	2520
CATCTGTGCG	GTATTTTACA	CCGCATATAT	GGTGCCTCT	CAGTACAATC	TGCTCTGATG	2580
CCGCATAGTT	AAGCCAGTAT	ACACTCCGCT	ATCGCTACGT	GACTGGGTCA	TGGCTGCGCC	2640
CCGACACCCG	CCAACACCCG	CTGACGCGCC	CTGACGGGCT	TGCTGTCTCC	CGGCATCCGC	2700
TTACAGACAA	GCTGTGACCG	TCTCCGGGAG	CTGCATGTGT	CAGAGGTTTT	CACCGTCATC	2760
ACCGAAACGC	GCGAGGCAGC	TGCGGTAAAG	CTCATCAGCG	TGGTCGTGAA	GCGATTACAA	2820
GATGTCTGCC	TGTTTATCCG	CGTCCAGCTC	GTTGAGTTTC	TCCAGAAGCG	TTAATGTCTG	2880
GCTTCTGATA	AAGCGGGCCA	TGTTAAGGGC	GGTTTTTTCC	TGTTTGGTCA	CTGATGCCTC	2940
CGTGTAAGGG	GGATTTCTGT	TCATGGGGGT	AATGATACCG	ATGAAACGAG	AGAGGATGCT	3000
CACGATACGG	GTTACTGATG	ATGAACATGC	CCGGTTACTG	GAACGTTGTG	AGGGTAAACA	3060
ACTGGCGGTA	TGGATGCGGC	GGGACCAGAG	AAAAATCACT	CAGGGTCAAT	GCCAGCGCTT	3120
CGTTAATACA	GATGTAGGTG	TTCCACAGGG	TAGCCAGCAG	CATCCTGCGA	TGCAGATCCG	3180
GAACATAATG	GTGCAGGGCG	CTGACTTCCG	CGTTTCCAGA	CTTTACGAAA	CACGGAAACC	3240
GAAGACCATT	CATGTTGTTG	CTCAGGTCG				3269

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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 λ DE3 lysogen, which expresses T7 RNA polymerase under the control of the *lacUV5* promoter¹⁾. 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): *lacI, lacUV5-T7 gene 1, ind1, sam7, nin5*)

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

Stable at -80°C 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 μ g/mL ampicillin. The efficiency was confirmed to be greater than 2×10^6 cfu/ μ g.

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Product Usage

| Cloning of a gene to pETBA:

Below is the multiple cloning site of pETBA. To express a recombinant protein correctly, it is necessary to clone the gene of interest in frame with an N-terminal peptide of pETBA. The start codon of pETBA is boxed ATG in the below figure. Digest pETBA 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 pETBA 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. Recombinant plasmids derived from pETBA 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 TTCGATTTTCG 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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| 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 μ L 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 μ L of competent cells, use ≤ 5 μ L).
 - *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 μ l of the cells to a 37°C 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 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.

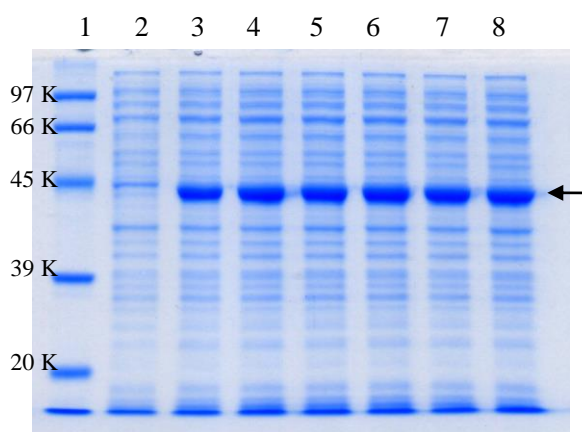
The IPTG concentration and induction time are general values. It is recommended to determine the optimal condition for the target gene expression.

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 μ l of 1 \times PBS buffer.
2. Mix an aliquot of the suspension (e.g., 100 μ l) with an equal volume of 2 \times 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 μ l) to SDS-PAGE. Western blot will help analyzing the expression of the target protein.
 - 2 \times SDS sample buffer : 2 % sodium dodecyl sulfate, 5 % 2-mercaptoethanol, 20 % glycerol, 0.02 % BPB, 62.5 mM Tris-HCl, pH6.8
 - 1 \times PBS buffer.: 20 mM sodium phosphate, 150 mM sodium chloride, pH7.4

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An arrow shows the expressed 44 KDa proteins.

Figure of protein expression from pETBA

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

- 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.

Reference:

- Studier, F.W. and Moffatt, B.A., *J. Mol. Biol.* 189 (1986) 113–130.
- Moffatt, B.A. and Studier, F.W., *Cell* 49 (1987) 221-227
- 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.

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Related Products:

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

Purchaser Notification:

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