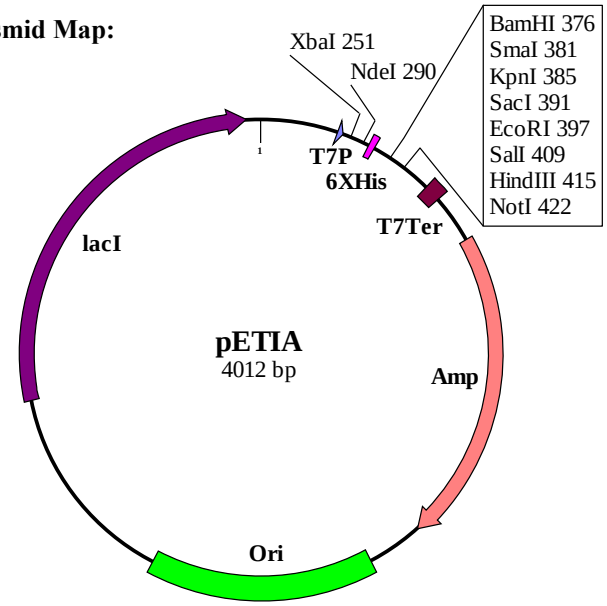
Product Name : pET Expression vector pETIA
Code No. : DV215
Size : 15 µg (lyophilized plasmid contains salt of TE buffer)
Storage : Store at -20ºC

Product Description:

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

| 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

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

| pETIA Sequence

DNA sequences are available on our web site.

https://www.biodynamics.co.jp/products/ex-pack/

| 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.

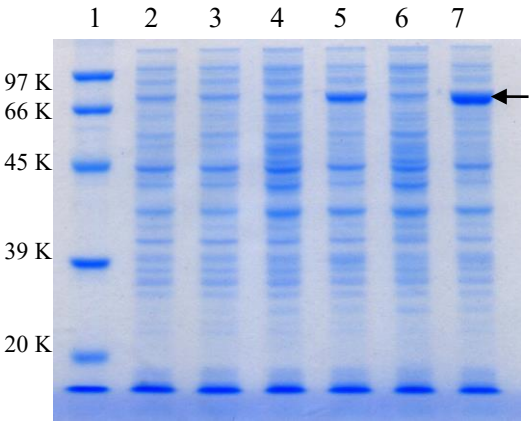
The IPTG concentration and induction time are general values. It is recommended to determine the optimal condition for the target gene expression.

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



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.

Related Products:

DS255	DynaCompetent Cells Zip BL21(DE3)
DS260	DynaCompetent Cells BL21(DE3)pLysS
DS230	DynaCompetent Cells JetGiga DH5α
DS520	AllView PAGE Buffer
DM660	DynaMarker Protein MultiColor Stable II
DS850 DS860	ONEPot Immunoassay Kit <OpenGUS Method>